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ANNALS OF BOTANY

VOL. L



# ANNALS OF BOTANY

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AND OTHER BOTANISTS

VOLUME L

With twenty Plates and four hundred and eight Figures in the Text

LONDON

HUMPHREY MILFORD, OXFORD UNIVERSITY PRESS  
AMEN HOUSE, WARWICK SQUARE, E.C.

EDINBURGH, GLASGOW, NEW YORK, TORONTO  
MELBOURNE, CAPE TOWN, AND BOMBAY

1936

PRINTED IN GREAT BRITAIN AT THE UNIVERSITY PRESS, OXFORD  
BY JOHN JOHNSON, PRINTER TO THE UNIVERSITY

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# Transpiration and Pressure Deficit.

## III. Observations by the Thermopile Method.

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With five Figures in the Text.

### INTRODUCTORY.

EXPERIMENTS described in previous communications (2, 3) have shown that when a pressure deficit (for definition see 1, p. 680) is artificially created, by enclosing the transpiring parts of cut leafy branches in a cylinder under increased pressure, the rate of transpiration decreases with increasing pressure deficit. The nature of the relation of transpiration rate to pressure deficit has been partially worked out (2, 3). The method employed however was indirect, the rates of transpiration being computed from rates of absorption after making allowances for changes in volume of the leaf cells. Confirmation of the results by experiments in which the transpiration rates were measured more directly therefore appeared desirable. Since however all the transpiring surfaces are enclosed within the pressure cylinder, none of the existing normal methods can easily be applied, especially when continuous series of readings are required.

It was frequently observed in the experiments quoted above that when the pressure was suddenly released (causing a great increase in transpiration rate) (3, p. 32), and the cylinder opened, the leaves were perceptibly cold to the touch as compared with normal leaves. In the series of experiments now recorded, therefore, a thermopile method has been exploited, the transpiration rates being estimated by their cooling effects on the leaf. The technical difficulties of the method are not great, both sets of junctions being enclosed within the cylinder (2, p. 220), one in contact with the experimental leaf surface and the other in most experiments in contact with a vaselined leaf at a standard distance of 5 cm. from the experimental junctions. The leads are brought out to a galvanometer through holes in the plug in the cylinder platform (2, p. 220). Some consideration is necessary of the exact significance of the readings obtained by such methods and of the relation expected to the rates of transpiration.

## THEORETICAL.

The relation of the observed galvanometer deflexions to the changes in transpiration rate will be evident from the following considerations.

When transpiration takes place a quantity of heat is absorbed depending upon the latent heat of vaporization and the quantity of water evaporated. The heat absorbed causes a reduction in temperature of the leaf by an amount depending upon the specific heat of the leaf and the mass of the leaf effectively associated with a given area of evaporating surface. The rate of loss of heat is thus directly proportional to the rate of evaporation. The rate of reduction of leaf temperature, however, depends also upon the mass and specific heat of the leaf, and also (especially in thick leaves) upon the thermal conductivity of the leaf. This latter factor will be very small for thin leaves, and can in any case be ignored for the present purpose for reasons which will become apparent. Reduction in leaf temperature will continue until the temperature difference between the leaf surface and the air is such that the rate of influx of heat from the air becomes equal to the rate of loss of heat by evaporation. The rate of influx of heat from the surrounding air at any given distance from the leaf is inversely proportional to the thermal conductivity of the air, and directly proportional to the temperature difference between the air at this point and the leaf surface.<sup>1</sup> The incoming heat will tend to heat the leaf to an extent depending upon exactly the same factors as those on which depends the extent of cooling by the heat lost, viz.:—the specific heat of the leaf, the mass of leaf effectively associated with unit evaporating surface, and the thermal conductivity within the leaf as far as this comes into play. For further purposes, therefore, all these latter factors may be left out of account, since the leaf temperature will become constant when the rates of heat gain and heat loss deciding it in conjunction with these factors become equal. Thus if  $T$  = the transpiration rate,  $c$  = the conductivity of the air,  $t_l$  = the leaf temperature and  $t_a$  = the temperature of a layer of air distant  $d$  from the leaf surface, when the leaf temperature is constant, the rate of heat loss = the rate of heat gain, or  $T$  = the sum of all the expressions,  $(t_a - t_l)c/d$ , with different corresponding values of  $t_a$  and  $d$  relating to all the different layers of air effective in heating the leaf. Supposing then that conditions are constant and transpiration is proceeding at a uniform rate, as soon as the leaf temperature has become reduced to the equilibrium value at which it remains constant, the rate of heat gain from the air by conduction will be equal to the rate of heat loss by evaporation, and the difference in temperature between the leaf and any given layer of air in its vicinity will

<sup>1</sup> Only heat transference by conduction need be considered. Radiation from the walls of the cylinder will be relatively very small, and any assistance by convection must be preceded by conduction from the layers of air convected. The method of heat gain, moreover, is in any case immaterial as long as it is proportional to the extent of cooling of the leaf.

be proportional to the transpiration rate. Since the galvanometer deflexions are (for small deflexions) proportional to the difference in temperature between the two series of thermo-junctions, on the leaf surface and at a given distance from the leaf respectively, the deflexions measured will be directly proportional to the transpiration rates required. It is important to realize, however, that this convenient state of affairs is only true when the readings are constant, that is to say, when the transpiration rate is constant and the leaf has also acquired the constant reduced temperature which satisfies the requirements of the thermodynamic equilibrium between the leaf and the surrounding air under the given (constant) conditions of rate of heat loss. At all other times, i.e. when changes in transpiration rate are taking place, the deflexions corresponding to temperature differences will represent these changes correctly in sense but will be altogether disproportionately exaggerated in magnitude.

This latter point will be more evident from the following considerations. Since it is only the cooling of the leaf through evaporation which leads to the temperature difference between the leaf surface and the air upon which influx of heat to the leaf depends, and since transference of heat by conduction as a result of this temperature difference requires time to take place, it is obvious that while, for instance, the transpiration rate is increasing and the leaf temperature falling, the processes (vaporization) making for heat loss by the leaf will keep in excess of those making for heat gain (influx through temperature difference). The latter being a result of the former must lag behind to an extent depending upon the rate of conduction of heat from the air to the leaf surface. Consequently as compared with the equilibrium values at which the deflexions are proportional to the transpiration rates, the deflexions and temperature differences at times of increasing transpiration will be greater than the actual transpiration rates, and for similar reasons during decreasing transpiration rates will be considerably too small. Both decreases and increases will be exaggerated in the same way. Incidentally, it may be pointed out that the discrepancies will be roughly proportional to the rates of increase or decrease. The exaggerated readings obtained during changes in transpiration rate therefore, though not proportional to the actual rates, have some value in that they are roughly measures of the rates of change. The actual rates can only be estimated when the readings have again become constant.

It might be argued that the galvanometer deflexions giving the differences between the leaf temperatures and the temperature of the air would not necessarily be expected to be measures of the rates of transpiration, on the grounds that if no transpiration were proceeding, the normal leaf temperature would not become equal to that of the air, but would be somewhat higher on account of respiration. Transpiration would then not be proportional to the difference between the temperatures of the leaf and the air, but to the difference between the actual leaf temperature and the temperature which the leaf would acquire if transpiration were reduced to

zero. This difference would be greater than that measured. Observed transpiration rates would be too small and percentage changes would be exaggerated.

To obviate this error comparison was made, not with the air, but with a vaselined leaf, and this only using fully expanded leaves in which the respiration would have the lowest possible values. Experiments moreover show that there is no appreciable heating effect of respiration in the types of leaf used, since neither with vaselined leaves nor leaves in saturated air could it be shown that the leaf temperature ever rose consistently above that of the air. Even when leaves were tested immediately after vaselining, and there could have been no time for respiration to be materially reduced through depletion of intercellular oxygen, the result was the same. It was in fact only with difficulty that leaves could be vaselined sufficiently well to allow their temperatures to come up to those of the air. These points are brought out by the results of preliminary experiments (p. 11) and show clearly that there can be no significant error due to respiration, especially with the method of control used involving comparison with a vaselined leaf.

When the method is used to compare the transpiration rates at different air pressures a further point arises, since the deflexions, as will be seen from the equation on p. 2, depend directly upon the thermal conductivity of the air. Should the thermal conductivity be increased a given transpiration rate would only give rise to a smaller temperature difference and therefore a smaller deflexion. Actually however the thermal conductivity of air is throughout a sufficiently wide range practically independent of the pressure. Although the number of molecules per unit volume increases with pressure, the mean free path of each is reduced in the same proportion and the two effects cancel one another. The method is therefore not rendered inapplicable to the problem by changes in thermal conductivity of the air.

It may further be pointed out that in actual experiments the leaf temperature deciding the deflexion is that of certain points on the leaf which are in contact with the grid of wires forming the series of thermo-junctions. Since the thermal conductivity of the leaf-tissues must at all times be considerably greater than that of the air, and that of the wires of the grid is enormously so, it follows that an important means of heat gain by the elements of the leaf surface immediately surrounding the points of contact with the thermopile grid will be by conduction through the wires of the grid. In view of the greater heat capacity of the metal as compared with the air, it is evident that a large proportion of the heat gained will be supplied from the heat capacity of the wires on the reverse side of the thermopile former (pp. 5, 6), and the rate of this supply will be entirely unaffected by pressure. The possibility of this contributory source of heat in no way affects the general arguments already put forward. The wires will eventually be re-heated

from the surrounding air but there will be considerable lag before this stage of the process sets in. There is therefore no reason to suppose that constant deflexions will not be proportional to the transpiration rates. This is also shown by experiments (p. 11, Exp. O).

#### APPARATUS AND METHODS.

It is desirable that the thermopile should detect the difference between the temperature of the leaf surface and that of the air at approximately 5 cm. distance, measured in the plane of the leaf rather than normally away from its surface, since at such points the air temperature will be least affected by the cooler vapour diffusing away from the leaf surface, and the temperature difference will be as large as possible for a given distance from the leaf.

The type of thermopile used therefore is that shown in Fig. 1. It has twenty-five pairs of junctions and is made by winding appropriately cut lengths of 36 s.w.g.<sup>1</sup> copper and 36 s.w.g. iron wire on a specially shaped former of  $\frac{1}{8}$  inch sheet fibre. The two sets of junctions come opposite holes or 'windows' OO, in the former, against one of which the experimental leaf is placed and held lightly in position by means of thin rubber bands. The iron wire (between the lines NN in the diagram) is bare, the copper wire insulated except opposite the windows immediately against the junctions. The fibre former is made first. The most convenient size is about 10 cm. square, each of the windows measuring  $2.7 \times 1.0$  cm. The windows are 4 cm. apart, 5 cm. between the centres. Holes are drilled to take two 4 B.A. terminals at A and B, and the four rows of small holes 1 mm. respectively from the four long sides of the windows are pressed through the fibre at previously marked points by means of a short piece of steel needle held in a lever feed drilling machine. These holes should be only just large enough to admit the 36 s.w.g. wires (0.025 cm. including insulation) and evenly spaced at 1 mm. apart. Two  $\frac{1}{8}$  inch (or 3 mm.) holes are drilled at C and D for the return windings. The whole former is then filed smooth where necessary, the edges of the windows being bevelled off at  $45^\circ$  on the reverse side. Keyhole slots, JJJJ, and KKKK, are provided at the sides of the former at the same height as the windows for the insertion of thin rubber bands, L, M, as shown, for securing the leaves. The former is then thoroughly dried by baking.

To wind the former with wire, twenty-five 5.8 cm. lengths of iron wire are cut first and the last millimetre of each at one end only bent round into a hook. The winding is started by inserting a terminal at A and carrying a short length of insulated (double silk-covered) copper wire from the terminal to C on the upper face of the former, down through C, up behind the former to the uppermost hole, E, on the left of the left-hand

<sup>1</sup> Diameter = 0.0076 inch, = 0.0193 cm.

window, up through E and across the window to 1 mm. beyond the mid-line where it is cut off and the last millimetre hooked. A prepared hooked length of iron wire is interlocked with it in the centre of the window and passed down through F, along the back of the former to G, up through G

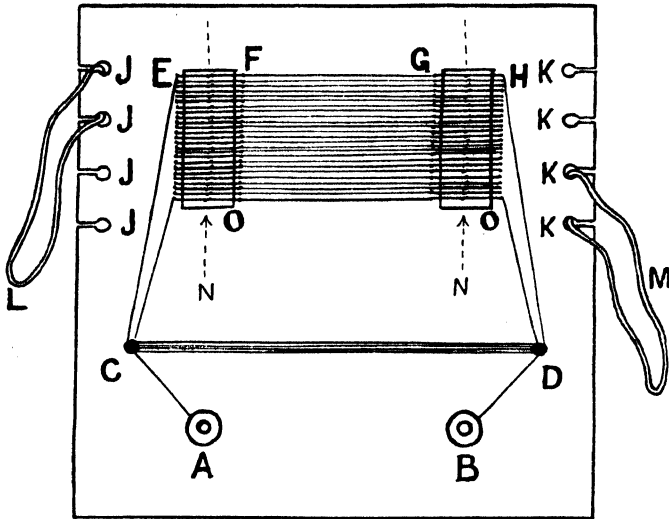


FIG. 1. Diagram of thermopile. For explanation see text, pp 5, 6. (For clearness most of the windings on the back of the former are omitted, except between the windows, o o.)

and across the right-hand window to 1 mm. beyond the mid-line where it is cut off if necessary and the last millimetre hooked. A length (approximately 18 cm.) of the insulated copper wire is then interlocked with this in the centre of the window and passed down through H, along the back of the former to D, up through D, along the face to C, down through C and up through the second hole at E where it is cut off and hooked in the centre of the window as before. The same cycle of operations is then repeated in exactly the same manner until the twenty-five pairs of junctions are in place, the last turn which is passed up through D being secured to a terminal entered at B. Throughout the winding the wires should be kept as taut as the hooks will allow. When all the wire is in place a trace of solder is run on to each of the junctions, using a resinous (not acid) flux. The tension of the windings may then be increased by placing two small strips of wood, pieces of match-stick being very suitable, between the former and the windings on the reverse side at the mid-point between the windows, and working these strips as near as possible to the sides of the two windows respectively, thus increasing the tension in the wires. The whole apparatus, including all the windings except where they cross the windows, is then thoroughly treated with shellac varnish and baked, this being a very necessary precaution, not only for the sake of

insulation, but also to prevent warping of the fibre in damp air. When the varnish has hardened, any odd ends of wire at the junctions are trimmed off and superfluous solder removed if necessary in order to avoid unnecessary heat capacity. The junctions are then thoroughly washed with ether on a brush to remove traces of flux. Next they are washed in the same way with absolute alcohol, after which tap-water is allowed to trickle over them for some hours to remove traces of acids or other impurities which are otherwise apt to set up E.M.F.'s at the junctions. After a further washing in distilled water, the junctions are again treated with absolute alcohol and ether and allowed to dry. The thermopile is then ready for use, but must frequently be similarly cleaned between experiments. The sensitivity of such a thermopile with the galvanometer used was of the order of fifty scale divisions per  $1^{\circ}\text{C}$ .

#### EXPERIMENTAL METHODS.

In pressure cylinder experiments the plant was set up in the cylinder in exactly the same manner and with exactly the same precautions as already described for other methods (2, p. 224), except that the experiments were conducted in the dark, and all leaves were detached from the branch except a single experimental leaf. The second leaf from the same node as the experimental leaf was vaselined and used for the control. The thermopile was held in a special clip inside the cylinder, the base of the clip being conveniently embedded in the wax seal. The experimental leaf was then placed with its lower surface against the face of the thermopile in such a position as to cover one of the windows and to come into light contact with the corresponding junctions. It was held lightly in this position by thin rubber bands, the band from each pair of key-hole slots being passed across the leaf and slipped into the corresponding slots on the opposite side of the former. Transpiration through the window from the experimental area should be unimpeded, since the conductors are sufficiently thin as compared with their spacing to offer only a negligible resistance to diffusion. For the sake of uniformity the edge of the leaf is brought just to the mid-line between the windows in all experiments. Rubber-covered flexible leads are then connected with the two terminals of the thermopile and allowed to pass out through two separate holes, which will only just admit them, drilled through one half of the split plug of the seal (2, p. 220). They pass further through corresponding holes in the metal parts of the plug to the D'Arsonval mirror galvanometer. The wires are knotted on the inside of the cylinder just above the wooden part of the plug in order to prevent their passing further through the plug under the pressure. During experiments the knots are buried in the wax of the wax seal which is made in the usual way (2, p. 222).



As soon as the setting up is complete the interior of the cylinder is darkened by means of a light-tight cover placed on the top of the cylinder in order to eliminate direct effects of light upon the thermopile. The deflexions become constant after a half to one hour. This time is evidently necessary for the disappearance of E.M.F.'s set up before enclosure of the thermopile in the cylinder since no apparent change in the condition of the stomata was detected by the injection method within two and a half hours after darkening. When the deflexion has remained constant for half an hour the reading is taken as the 'initial rate of transpiration at atmospheric pressure' recorded in the Tables. The increased pressure is then applied slowly, approximately one minute being required for the full pressure to be reached. The deflexions during half an hour at the increased pressure are then followed up at one-minute intervals. The pressure is then slowly released, the release also being allowed to take approximately one minute. In some experiments the deflexions were again followed up at one-minute intervals during the following half hour at atmospheric pressure.

An exactly similar procedure is followed in the control experiments (p. 8). The readings obtained in the control experiments are then subtracted each from the corresponding readings in the actual experiments, the differences giving the changes in transpiration rate resulting from the increases and decreases in pressure deficit.

#### CONTROL EXPERIMENTS.

Certain complications are introduced as a result of the fact that the air let into the cylinder to increase the pressure may not be at exactly the same temperature as that originally in the cylinder. Moreover, the entering air falls in temperature as it expands. Similarly on release of the pressure the contained air cools during its further expansion. Both increase and decrease in pressure will also necessarily be accompanied by currents of air within the cylinder. For all these reasons it is only to be expected that during changes in pressure in the cylinder the contained air will not be at a uniform temperature and differences in temperature of the two sets of thermo-junctions, and consequently deflexions of the galvanometer, will result quite independently of those set up by transpiration from the leaf. The leaf itself, moreover, may play a part in shielding one set of junctions more than the other from currents of air at different temperatures. Control experiments are therefore necessary to determine in how far the experimental deflexions are due to transpiration and transpiration changes.

Two possible methods of control suggest themselves.

(1) The difference in temperature between the transpiring leaf surface and the surrounding air may be compared with that between a vaselined leaf and the surrounding air. For this purpose readings may first be obtained

with a leaf on the experimental window of the thermopile and nothing on the other; and from the results may be subtracted the readings obtained in a control experiment with a vaselined leaf on the experimental window and nothing on the other. The differences should give the deflexions due to lowering of leaf temperature by transpiration alone. Or

(2) The surface temperature of the transpiring leaf may be directly compared with that of a vaselined leaf. In this case readings are first obtained with an experimental leaf on one window and a vaselined leaf on the other, and from the results are subtracted the corresponding readings obtained in a control experiment with nothing on either window. This latter method might be expected to be the better, since the two sets of junctions would be equally protected or unprotected from air currents in all cases. The effects of these may, moreover, be completely eliminated by taking cognizance of experimental readings only at such points in the experiment as the control readings are at zero or negligibly small. Here again the differences should be due to transpiration alone. It will be observed that the effects of respiration are allowed for in both types of experiment, inasmuch as the lowering of leaf temperature by transpiration is measured relative, not to the air temperature, but to the possibly higher temperature of a vaselined leaf. (The vaselined leaf used was always the pair to the experimental leaf from the same node.)

Both types of experiment have been performed, the results being very similar in the two cases. Further experiments have shown, moreover, that the results are also the same if, instead of a vaselined and an unvaselined leaf, vaselined and unvaselined portions of one large leaf are used opposite the two sets of junctions. This method, though providing a sounder control in some ways has not been used further since it involves using leaves that are so large that an undesirably large total area of leaf surface has to be enclosed within the cylinder, and this may lead to increase in the humidity of the contained air through relative inefficiency of the drying agent as already discussed (2, p. 226).

#### PRELIMINARY EXPERIMENTS.

All types of preliminary experiment show erratic readings on first setting up, no doubt owing to the effects of light and draughts upon the junctions before they are enclosed in the cylinder. The initial erratic period, however, seldom exceeds fifteen minutes.

Blank experiments with nothing on either window show the usual erratic readings for a few minutes, but the zero is reached within ten to fifteen minutes both in dry and saturated air, and is then maintained constant (Table I, A and B).

Dry filter paper on one window causes no significant deflexion (Table, I, C).

Wet filter paper on one window and either dry filter paper or nothing on the other give in dry air a deflexion gradually decreasing to zero in the course of a few hours as the paper dries out (Table I, D, E).

In air which is as nearly saturated as possible by pads of wet cotton-wool in the cylinder, wet filter paper gives as a rule a very small deflexion indicating some evaporation, evidently on account of the impossibility of completely saturating the air by this means (Table I, F), though in two experiments out of six a true zero was attained within three quarters of an hour and maintained for a further half hour. By passing cooled water vapour from a steam heater into the slightly raised cylinder by means of a rubber tube, true zeros could readily be obtained but not maintained on account of draughts. Using a sealed bell-jar instead of the pressure cylinder the readings came almost to zero, using *Acer* leaves in thirty minutes, provided they were completely darkened, a very small amount of transpiration, corresponding to 0.1–0.3 of a scale division however still proceeding, evidently on account of imperfect saturation (Table I, G).

TABLE I.

*Giving the Results for One Typical Experiment of each Type of Control Mentioned in the Text (pp. 9–14). Similar results were obtained with three experiments of each type.*

All the figures in the table give galvanometer deflexions in scale divisions, read at one minute intervals. The (usually erratic) deflexions of the first fifteen minutes after setting up have been ignored except where otherwise stated.

Unless otherwise stated the temperature of the experimental junctions is *below* that of the control junctions as when the difference is due to evaporation. Readings marked – indicate that the temperature of the experimental junctions is *above* that of the control junctions as when the difference is due to draughts from the inlet, etc., or to respiration.

A. Blank experiment in dry air, (From beginning).

17 7 4 2 1 0 –0.5 –0.5 –0.2 0 0 0 0 0 0 0 0 0 0 0

B. Blank experiment in saturated air. (From beginning).

0 0.1 0.1 0.1 0.0 0.05 0.05 0 0 0 0 0 0 0 0 0 0 0 0 0

C. Dry filter paper in dry air. Control: nothing.

1 0.5 0.0 0.5 0.0 –0.5 0 0 0 0.5 0 0 0 0 –0.5 0 0 0 0 0

D. Wet filter paper in dry air. Control: nothing.

12 10 8 7 6.7 6.3 6.0 5.8 5.5 5.0 4.5 4.1 3.8 3.5 3.3 3.1 3.0 2.8  
2.5 2.3 2.1 2.0 1.8 1.5 1.5 1.5 1.5 1.4 1.4 1.4.....0

E. Wet filter paper in dry air. Control: dry filter paper.

15 13.5 12 11 10 9 8.5 7.5 7.0 6.5 6.0 5.5 5.0 4.8 4.5 4.0 3.5 3.3  
3.1 3.0 2.9 2.7 2.5 2.4 2.3 2.3 2.3 2.3 2.3 2.3.....0

F. Wet filter paper in saturated air. Control: nothing.

–0.2 –0.2 –0.2 –0.2 –0.1 –0.1 0.0 0.1 0.1 0.1 0.1 0.2 0.1 0.1 0.1  
0.1 0.1 0.1 0.1 0.1 0.1 0.0 0.0 0.0 0.0.....0.0

TABLE I (*continued*).

G. Leaf of Acer in saturated air in bell jar. Control: nothing.

-5.0 -2.0 -1.0 -0.3 -0.1 0.0 0.1 0.2 0.3 0.3 0.3 0.3 0.3 0.3  
0.3.....0.3

H. Very young leaf in saturated air in the dark. Control: nothing.

-0.3 -0.3 -0.3 -0.2 -0.2 -0.2 -0.1 -0.1 -0.1 -0.1 -0.1  
-0.1 -0.1 -0.1 -0.1 -0.1 0.0 0.0 0.0 0.0 0.0; 20 min. later, 0.1 and  
constant at 0.1 for one hour.

K. Injured leaf in saturated air in the dark. Control: nothing. (From beginning.)

0.0 -1.0 -1.8 -2.9 -4.1 -5.0 -6.0 -6.9 -4.3 -3.0 -1.9 -0.8  
-0.2 0.0 0.3 0.5 0.7 0.9 1.0 1.1 1.2 1.3 1.2 1.1 1.0 1.0 1.0

L. Acer leaf in saturated air in the dark. Control: nothing. (Readings at five-minute intervals from the beginning.)

14.5 3.6 0.9 0.8 0.7 0.7 0.8 0.9 0.9 0.9 1.0 1.0 1.0 1.0; on opening to  
air, 6.8.

M. Acer leaf as in L.

0.0 -0.3 0.2 0.9 1.4 1.7 1.8 1.7 1.6 1.5 1.4 1.45 1.4 1.4 1.4 1.4  
1.4 1.3 1.3 1.35 1.4 1.35 1.4 1.35 1.3 1.3 1.2 1.1 1.1

N. Vaseline'd Acer leaf in dry air. Control: nothing. (From beginning.)

7.0 3.5 1.9 0.6 0.2 0.0 0.2 0.4 0.2 -0.1 0.1 0.2 0.3 0.4 0.4 0.4  
0.3 0.3 0.4 0.4 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3

O. Comparison of deflexions with rates of water loss by weighing.

Half hour periods:	1.	2.	3.	4.
Rates of loss in gm./hr.	0.2610	0.2580	0.2400	0.1578
Mean deflexions	4.00	4.28	3.80	2.40
Ratios (deflexion/rate)	15.3	16.2	15.8	15.2

Experiments on very young leaves in saturated air in the dark showed the leaf temperature to be slightly higher than that of the surrounding air (Table I, H). The same is true of experiments on leaves injured by repeated piercing with a thick needle (Table I, K). In this case the leaf temperature rises above that of the air for the first ten minutes, and later falls back to slightly below it on account of the increased evaporation. It will be noted that in experiments where the leaf temperature is found to be above that of the air the excess is no measure of the respiration rate, since even in saturated air it will lead to increased evaporation by which it is automatically reduced. Respiration rates computed on this basis would therefore appear too small. The respiration correction is therefore computed from experiments on vaselined leaves. With leaves such as those used in the experiments, however, the respiration rates could not be sufficient to vitiate the results, since with these it could never be detected (after the initial period as in I, G) that the leaf temperature rose above that of the surrounding air, even when the air was as nearly saturated as possible, and the leaves were in complete darkness (Table I, L, and M).

Experiments with vaselined leaves in dry air invariably showed a very slightly lowered leaf temperature, but it is doubtful whether this was significant (Table I, N).

The control experiments taken together show that the experimental deflexions measured later must have been due to the effects under investigation, since (a) leaves of the type used have a negligible respiration correction, (b) non-evaporating surfaces give no deflexion at the points in the experiments of which use is made, (c) wet-surfaces no longer give significant deflexions when evaporation is eliminated by enclosure in saturated air, and (d) erratic effects are over before the time the essential readings are taken.

In addition to these control experiments attempts were also made to calibrate the thermopile in terms of transpiration rates by comparison of the galvanometer deflexions (a) with simultaneous readings of a potometer, and (b) with the rates of transpiration as found by weighing, the thermopile being mounted on the stirrup of the balance pan.

Potometer readings throughout a large number of experiments showed no relation whatever to the deflexions. This is evidently largely due to the different rates of transpiration of different leaves, since there is some measure of agreement when all the leaves are stripped off the branch except the experimental leaf used on the thermopile. With only one leaf and efficient shading from draughts and direct light the variations are at least usually in the same sense, but the different amounts of lag between the changes in transpiration rate and the consequent changes in absorption rate render the method useless for the purpose of calibration. It was therefore not pursued further.

The weighing method, on the other hand, provided that only one leaf was used, and that this was adequately shielded from the light, and that the whole apparatus was kept in a large transpiration chamber perfectly free from draughts, gave moderately good agreement. The method of experiment was as follows. A piece of branch approximately 30 cm. long was stripped under water of all the leaves except one, and the scars were sealed with wax. The branch was dried, and its base was placed in a small vessel of water. A thin layer of oil was placed on the water, and the whole placed on the balance pan with the leaf against the experimental window of the thermopile. The latter was mounted on the stirrup, and connected by very flexible leads to the galvanometer. An hour was then allowed for the branch to become saturated and the transpiration to become constant. At the end of this time the system was weighed and the deflexions recorded at regular intervals. After another half hour the water supply was removed, and the base of the branch sealed with wax in order to cause a falling off in the rate of water loss, and the same procedure was continued. The rates of loss for four successive half hours in a typical experiment, together with the corresponding deflexions are given in

Table I, O, the first readings being those before the removal of the water supply. It will be seen from the ratios in the last line that the agreement in this particular case is within 7 per cent., though in other experiments it was only within 10 per cent. This is probably quite as good as could be

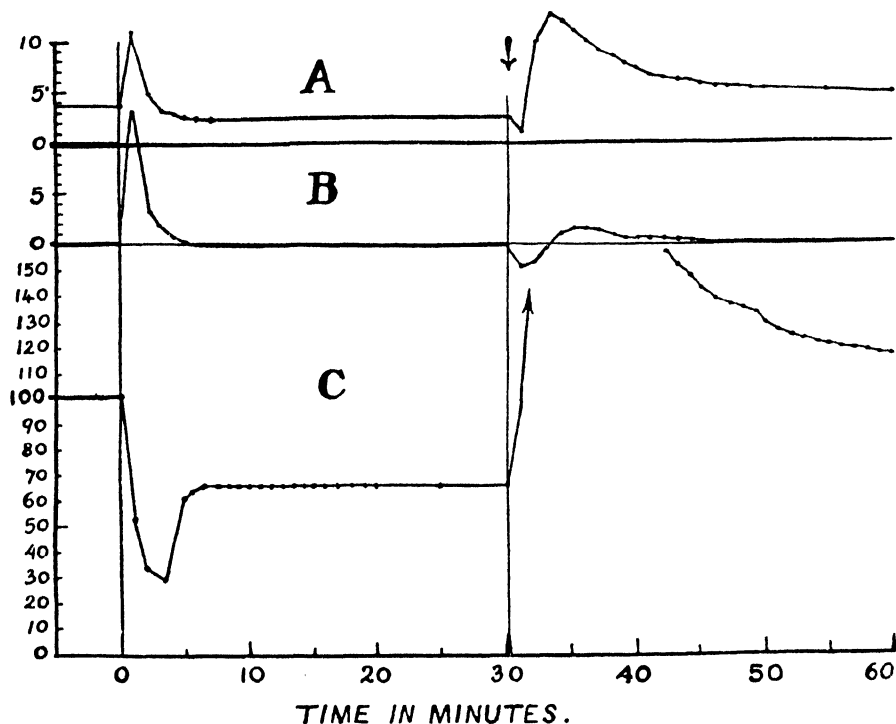


FIG. 2. Graphs of a complete typical 'Series I' experiment as detailed in Table II to show general behaviour of readings. A. Experimental deflexions in scale divisions with normal leaf on experimental window of thermopile and nothing on the control window. B. Deflexions in control experiment with vaselined leaf on experimental window and nothing on the control window. C. Apparent transpiration rates (differences between A and B) expressed as percentages of the initial rate at atmospheric pressure. These values are only truly proportional to the actual transpiration rates when constant. It will be noted that only the constant values obtained at the ends of the periods under pressure are used for the purpose of estimating the relative transpiration rates at different pressures.

The increased pressure (50 lb./in. in this experiment) is applied at the vertical line corresponding to 0 on the time axis. The lines in the space to the left of this vertical line indicate the initial constant rates attained before the pressure was applied. The pressure is released at the point indicated by the arrow, thirty minutes after the point at which it was raised. The control curve shows at what point the experimental curve becomes no longer affected by the initial erratic effects of inlet and outlet of air (cf. p. 8).

expected, as the transpiration chamber used was fitted with sashes, and therefore liable to admit a certain amount of draught. Light, moreover, could not be completely excluded. The experiments taken together definitely suggest, however, that in the pressure cylinder where there is no possibility whatever of leakage to either draughts or light the agreement would be easily within 5 per cent., and certainly within the 7 per cent.

attained outside. While it is no doubt to be regretted that there is no possible method of proving this more directly or conclusively, it may be pointed out that the differences dealt with in the actual experiments, as will be seen below, are of such an order that the point in question would

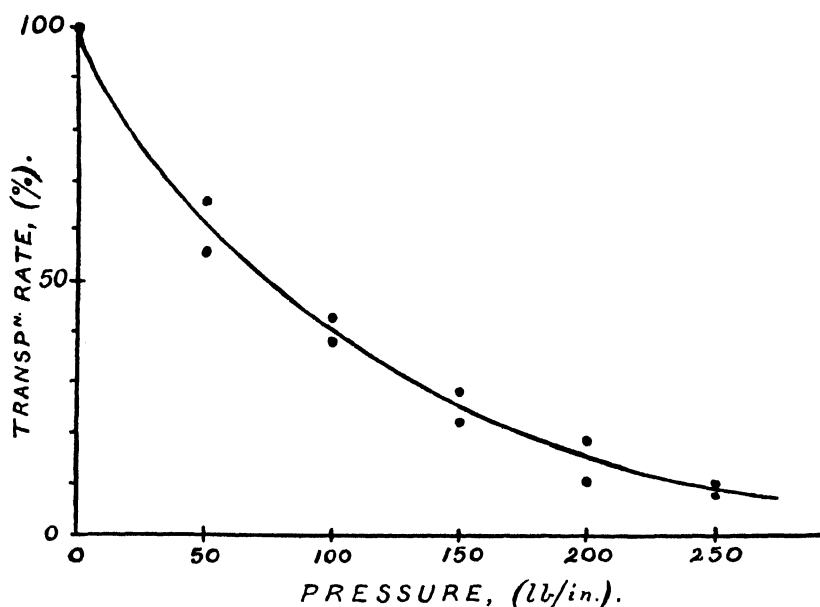


FIG. 3. Graph plotted from the figures in Table III to show the relation between transpiration rate and pressure deficit as determined by the method of Series I.

It may be noted that the higher points throughout, i.e. those lying above the curve of nearest approach, are for leaves with lower initial transpiration rates. The low points are for leaves with higher initial rates. It is doubtful, however, whether in this case the differences between the initial rates were always sufficient to render this apparent relation significant.

still be proved if the error were more than five times the maximum error indicated by the control experiments.

#### EXPERIMENTS AND EXPERIMENTAL RESULTS.

Two series of experiments have been performed: Series I by the method of control (1) (p. 8), and Series II by the method of control (2) (p. 9). The results are substantially the same by both methods, though those obtained by method (1) are slightly higher throughout than those obtained by method (2). The reason for this difference is not apparent unless it should be that slightly older leaves were used at the time the Series I experiments were performed than in the following year when the Series II method was principally used. This would account for the difference, but this effect of age (p.20) was not realized at the time of carrying out the Series I experiments.

In order to save space the detailed results are only given for one experiment in each series (Tables II and IV and Figs. 2 and 4). These will serve to show the general trend and behaviour of the readings, though in each case use is only made of the initial and ultimate constant readings

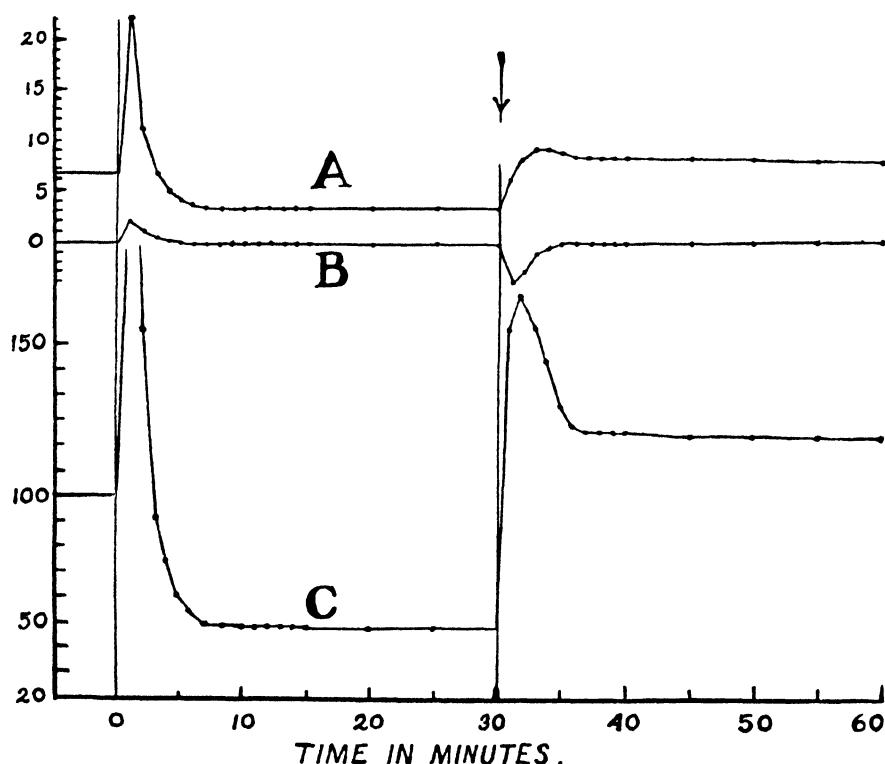


FIG. 4. Graphs of a complete typical 'Series II' experiment as detailed in Table IV to show the general behaviour of the readings. A. Experimental deflexions in scale divisions with normal leaf on experimental window of thermopile and vaselined leaf on the control window. B. Deflexions in blank control experiment with nothing on either window. C. Apparent transpiration rates (differences between A and B) expressed as percentages of the initial rate at atmospheric pressure. These values are only truly proportional to the actual transpiration rates when constant. Only the constant values obtained at the ends of periods under pressure are used for the purpose of estimating the transpiration rates at different pressures.

The increased pressure (50 lb./in. in this experiment) is applied at the vertical line corresponding to 0 on the time axis. The lines in the space to the left of this vertical line indicate the initial constant rates attained before the pressure was applied. The pressure is released at the point indicated by the arrow, thirty minutes after the point at which it was raised. The control curve shows at what point the experimental curve becomes no longer affected by the initial erratic effects of inlet and outlet of air (cf. p. 8).

for calculating the rates of transpiration under deficits. The summarized results of all the Series I experiments, giving the essential readings only, are collected together in Table III and plotted graphically in Fig. 3. Those of the experiments of Series II are given in Table V and plotted in Fig. 5. The experimental material throughout was *Acer pseudoplatanus*.



TABLE II.

*Detailed Results of a Typical Experiment on Acer pseudoplatanus  
by the method of Series I (p. 14).<sup>1</sup>*

Experiment 1.					
Time from beginning of expt. in min.	Pressure in lb/in.	Experi- mental deflexion.	Deflexion in control expt.	Difference.	Transpiration rates as percentages of the rates at atmospheric pressure. <sup>2</sup>
0	0	3.8	0.0 <sup>3</sup>	3.8	100
1	50	11.0	13.0	2.0	52.6
2	50	4.8	3.5	1.3	34.2
3	50	3.1	2.0	1.1	29.0
4	50	2.9	0.6	2.3	60.5
5	50	2.6	0.2	2.4	63.1
6	50	2.5	0.0	2.5	65.7
7-30	50	2.5	0.0	2.5	65.7
31	0	1.0	-2.5	3.5	92
32	0	10.0	-2.0	12.0	316
33	0	12.5	-0.5	13.0	342
34	0	12.0	1.0	11.0	290
35	0	11	1.3	9.7	255
36	0	10.0	1.1	8.9	234
37	0	9.0	1.0	8.0	210
38	0	8.3	0.7	7.6	200
39	0	7.5	0.5	7.0	184
40	0	7.0	0.5	6.5	171
41	0	6.5	0.4	6.1	160
42	0	6.2	0.3	5.9	155
43	0	6.0	0.2	5.8	153
44	0	5.8	0.2	5.6	148
45	0	5.5	0.1	5.4	142
46	0	5.3	0.0	5.3	139
50	0	4.9	0.0	4.9	129
60	0	4.5	0.0	4.5	118

## DISCUSSION.

It will be observed from Tables III and V and Figs. 3 and 5 that increase in the pressure deficit causes a decrease in the transpiration rate (cf. 2, p. 233, and 3, p. 547), the falling off being relatively more rapid for the smaller deficits (cf. 3, p. 547), so that the curves are convex towards the origin. Release of the pressure (decrease of the deficit) causes a disproportionate increase in the transpiration rate (cf. 2, p. 233 and 3, p. 552), which later falls back to normal (Tables II and IV and Figs. 2 and 4).<sup>4</sup> (Cf. 3, p. 553).

<sup>1</sup> These results are plotted graphically in Fig. 2.

<sup>2</sup> These figures are only expected to be numerically correct when constant (see p. 3), and only the constant values (shown also to be correct by the control deflexions) are utilized for the calculation of the transpiration rates under pressure as given in Tables III and V.

<sup>3</sup> In many experiments of this type small positive deflexions of vaselined controls were observed but not taken into account, as they could only indicate slight evaporation in spite of vaselining. The controls were only intended to show up errors due to respiration (if any) and to air movements during inlet and outlet of air.

<sup>4</sup> This effect was definitely observed in all experiments, but the figures obtained for the increased

At first sight it would appear from Fig. 5 that the margin of error, especially in the readings of percentage rates at the lower deficits, was very great, and the agreement between the values from different experiments on the same deficit was poor. A closer scrutiny of the Table on which the

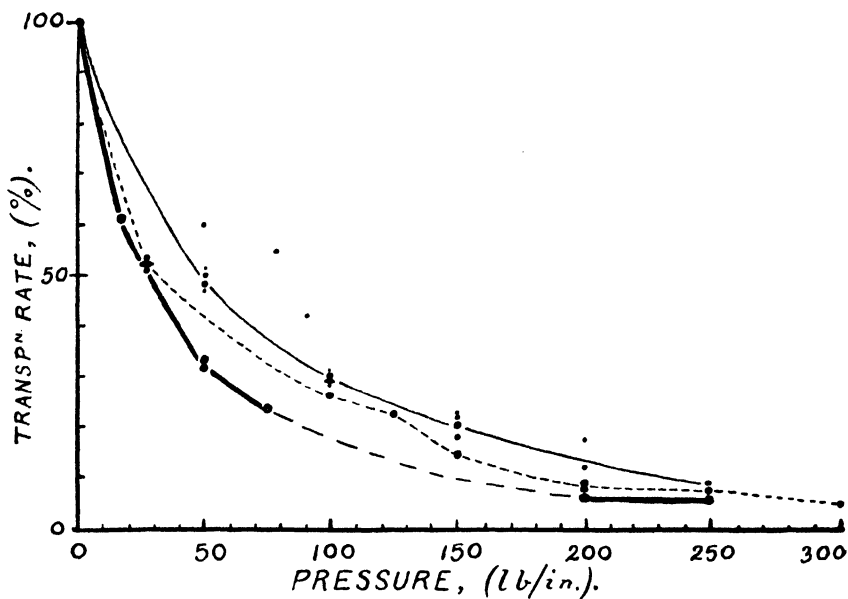


FIG. 5. Graphs plotted from the figures in Table V to show the relation between transpiration rate and pressure deficit as determined by the method of Series II. Taken as one series of points, the curve is markedly convex towards the origin. The graph also illustrates the different effects of pressure upon leaves with different initial transpiration rates. For this purpose the dots on the graph have been made roughly proportional in size to the initial rates of transpiration. Thus the largest points, joined except between 75 and 200 lb./in. by the heavy continuous line refer to leaves with initial rates of 15 units or over. These leaves are most affected by pressure, and the curve is therefore the lowest. The next smaller points, joined by the dotted line, refer to leaves with initial rates of between 10 and 15 units, and form the next higher curve. The remaining points, joined by the thin continuous line, refer to leaves with initial rates of less than 10 units and form the highest curve. The discordant points lying above the highest curve all refer to leaves with very low initial rates, two of them having initial rates of less than 5 units.

It will be noted that transpiration does not appear to cease entirely at any pressure investigated.

curves are based however (Table V), will show that the apparent discrepancies are mainly due to a lack of physiological uniformity in the material which cannot be detected in selecting uniform samples by eye. Thus it will be observed that the more rapidly transpiring leaves are nearly always considerably more affected by a given deficit than are those which originally only transpire more slowly. The experiments in this series have accordingly been re-numbered and arranged in the Table within each group on one deficit in ascending order of initial transpiration rates at

rates would not be numerically accurate since the readings did not become constant again until the original rates were reached (cf. p. 3). Except in the experiments detailed in Tables II and IV and Figs. 2 and 4, therefore, they have not been given in the tables.

TABLE III.

*Summarized Results of all Experiments of Series I.<sup>1</sup>*

Expt. No.	Pressure lb./in.	Initial rate at atmospheric pressure. (Scale divs.)	Rate under pressure. (Scale divs.)	Rate under pressure as percentage of initial rate at atmospheric pressure.
1 <sup>2</sup>	50	3.8	2.5	65.7
2	50	7.2	4.0	55.5
3	100	4.8	2.0	41.7
4	100	4.2	1.6	38.1
5	150	4.5	1.2	27.7
6	150	5.4	1.1	20.4
7	200	3.9	0.7	18.0
8	200	4.0	0.4	10.0
9	250	4.5	0.4	8.9
10	250	8.2	0.6	7.3

TABLE IV.

*Detailed Results of a Typical Experiment on Acer pseudoplatanus by the Method of Series II (p. 14).<sup>3</sup>*

## Experiment 17.

Time from beginning of expt. in min.	Pressure in lb./in.	Experimental deflexion.	Deflexion in control expt.	Difference.	Transpiration rates as percentages of the rates at atmospheric pressure. <sup>4</sup>
0	0	6.5	0.0	6.5	100
1	50	22.0	2.0	20.0	308
2	50	11.0	1.0	10.0	154
3	50	6.4	0.5	5.9	91
4	50	5.0	0.2	4.8	74
5	50	4.0	0.1	3.9	60
6	50	3.5	0.0	3.5	54
7	50	3.2	0.0	3.2	49.2
8	50	3.2	0.0	3.2	49.2
9	50	3.2	0.0	3.2	49.2
10	50	3.1	0.0	3.1	47.7
15	50	3.1	0.0	3.1	47.7
20	50	3.1	0.0	3.1	47.7
25	50	3.1	0.0	3.1	47.7
30	50	3.1	0.0	3.1	47.7
31	0	6.0	-4.0	10.0	154
32	0	8.0	-3.0	11.0	169
33	0	9.0	-1.0	10.0	154
34	0	9.0	-0.3	9.3	143
35	0	8.8	0.0	8.8	135
36	0	8.2	0.0	8.2	126
37	0	8.1	0.0	8.1	125
38	0	8.1	0.0	8.1	125
39	0	8.1	0.0	8.1	125
40	0	8.1	0.0	8.1	125
45	0	8.0	0.0	8.0	123
50	0	8.0	0.0	8.0	123
55	0	8.0	0.0	8.0	123
60	0	7.9	0.0	7.9	122

For notes to Tables III and IV, see opposite.

TABLE V.

*Summarized Results of all Experiments of Series II.<sup>1</sup>*

Expt. no.	Pressure. in lb./in.	Initial rate at atmospheric pressure. (Scale divs.)	Rate under pressure. (Scale divs.)	Rate under pressure as percentage of initial rate at atmospheric pressure.
11	15	22.2	13.4	60.4
12	25	22.9	1.5	51.7
13	25	13.4	7.2	53.7
14	25	15.5	8.2	52.9
15	50	2.0	1.2	60.0
16	50	5.1	2.6	51.0
17	50	6.5	3.1	47.7
18	50	7.0	3.5	50.0
19	50	7.3	3.5	47.9
20	50	19.0	6.0	31.6
21	50	20.0	6.6	33.0
22	75	4.6	2.4	52.2
23	75	24.5	6.0	24.5
24	90	7.0	2.8	40.0
25	100	2.9	0.9	31.0
26	100	6.9	2.0	29.0
27	100	7.8	2.3	29.5
28	100	9.0	2.7	30.0
29	100	13.4	3.6	26.9
30	125	12.5	2.9	23.2
31	150	3.6	0.8	22.2
32	150	6.6	1.2	18.2
33	150	6.8	1.5	22.1
34	150	6.9	1.4	20.3
35	150	13.4	1.9	14.2
36	200	6.2	1.1	17.8
37	200	8.0	0.9	11.3
38	200	10.8	1.0	9.2
39	200	14.4	1.2	8.3
40	200	15.0	1.0	6.7
41	250	5.5	0.5	9.1
42	250	11.5	1.0	8.7
43	250	15.0	1.0	6.7
44	300	13.5	0.8	5.9

atmospheric pressure. Running down the numbers in the last column for each group, it can readily be seen that the higher the initial transpiration rate the smaller the rate under any given deficit as compared with the original rate. Out of the thirty-four experiments recorded in the Table

<sup>1</sup> Plotted graphically in Fig. 3.

<sup>2</sup> Detailed results of this experiment in Table II and plotted in Fig. 2.

<sup>3</sup> These results are plotted graphically in Fig. 4.

<sup>4</sup> These figures are only expected to be numerically correct when constant (see p. 3, and p. 16, footnote 2).

<sup>1</sup> Plotted graphically in Fig. 5.

only six (viz.;—nos. 13, 17, 20, 27, 28, and 32) fall slightly out of sequence, and these only in cases where the initial rates are not far removed from those in the neighbouring experiments. Every significant difference in initial rate is accompanied by a difference in the effect of a given deficit in the sense indicated, the more rapidly transpiring material being the most affected. The relevant physiological differences between the different materials are evidently related principally to the time which has elapsed since the opening of the bud, when the material is collected, and the position of the leaf on the year's growth. Older leaves and those nearer the centre of the year's growth appear to transpire less rapidly than the younger leaves or those near one end of the year's growth, especially those near the apex. This observation falls well into line with the hypothesis already put forward (3, p. 561) that the effect of a deficit is partly due to its causing an increase in the resistance of the protoplasm of the leaf cells. It might well be expected that the protoplasm of the older leaves would have become more 'set' and less plastic, or less capable of reversible changes in physical state, and therefore less susceptible to the changes which might be induced by deficits. Hence one might expect the higher *percentage* transpiration rates under pressure deficits of older and less rapidly transpiring leaves. The suggestion which is partially conjectural at present is obviously capable of further investigation.

Some attempt to sort out the experiments relating to leaves of different ages or physiological states has been made in the preparation of Fig. 5 from the Table (Table V). Thus the dots on the graph have been made of four different sizes, the sizes being roughly proportional to the initial transpiration rates. It can be readily seen that the largest dots (referring to leaves with an initial rate of fifteen units and over) form one consistent curve, the next smaller dots (ten to fifteen units) another, and so on. The points for leaves of approximately similar physiological states have been tentatively joined together in the figure in order to draw attention to this apparent grouping and to the improvement in the complexion of the whole series of readings which results from taking this factor of physiological state also into account.

Whether the figure be looked upon as a series of graphs for physiologically different materials or even as a less accurate single series of points intended to lie on one curve, however, the main conclusion already reached by the potometer and eosin methods (2, 3) is definitely confirmed. This is that an increase in pressure deficit causes a progressive falling off in the rate of transpiration, the falling off being relatively greater for the smaller deficits. The curves (Figs. 3 and 5) are definitely convex towards the origin. The thermopile method would certainly not be considered a good one if a better could be applied, but even if the error of the method were 35 per cent. (five times the calculated maximum for cylinder conditions),

and the readings for the lower deficits should all have been 35 per cent. higher than those found, and the readings for the highest deficits should have been 35 per cent. lower than those found, the curve would still have been slightly convex towards the origin. There can therefore be no doubt that the transpiration rates fall off most rapidly for the smaller deficits and less rapidly as the deficit increases. The experimental curves therefore differ substantially from those which would be expected on the assumption that the changes in transpiration rate only would be those resulting from changes in suction pressure of the leaf cells (3, p. 554) and in vapour pressure at their surfaces, the structural factors deciding the resistance to flow of water through the leaves remaining constant.<sup>1</sup> These curves, as already pointed out (3, pp. 561, 563), would be concave towards the origin. Inasmuch as the present results fully confirm the type of rate-deficit curve already obtained, they corroborate the suggestion already put forward that deficits cause a considerable increase in resistance on the part of the leaf cells.

There remains still the possibility to be considered that the results of deficits *per se* (as applied in nature) may not be as great as these and the preceding investigations would lead one to suppose, since an additional effect of the deficits applied after the manner of the experiments by an increase in the pressure of the surrounding air, will be to reduce the rate of diffusion of moist air away from the leaf, and therefore for this reason also to reduce the transpiration rate. The effect of this factor is now forming the subject of a further investigation, but although it appears that it may modify some of the numerical values obtained, it does not appear that it will lead to any marked modification of the fundamental conclusions reached on the subject of possible alterations in the resistance to flow. It may be pointed out, moreover, that some such effect as the increased resistance indicated by these experiments evidently must exist to account for the magnitude of the changes in transpiration rate which occur at different times of the day and with different conditions of the plant. Leaf water-content determinations, from which the changes in vapour pressure at the cell surfaces can be calculated, show that, even when stomatal action (if any) and the changes in the effective areas of the evaporating surfaces are taken into account, the changes in vapour pressure which are possible are not nearly sufficient to account for the observed range of transpiration rates. It appears then that an important factor in the regulation of transpiration rate is the protoplasmic resistance to the flow of water through the cells. These points, however, will be elaborated further in later communications. Before this, however, it is hoped that the premises may be still further substantiated by a gravimetric method of estimating the transpiration rates under pressure which was not hitherto considered

<sup>1</sup> The actual resistance, being due to viscosity, would of course vary considerably with the rate of flow.

experimentally possible. Modifications of the apparatus are now being designed for this purpose.

#### SUMMARY.

Measurements of the transpiration rates from the leaves of *Acer pseudoplatanus* when enclosed in a cylinder under pressure have been made by a thermopile method, involving the comparison of the temperatures of a transpiring leaf and a vaselined leaf under the same conditions.

The results indicate that when an increased 'pressure deficit' (difference between pressure in the conducting tracts and pressure at the leaf surfaces) is applied in this way the transpiration is reduced, the reduction being relatively greater for the smaller deficits. A decrease in the deficit causes a very large and immediate increase in transpiration rate.

The rate-deficit curve is definitely concave towards the origin. This implies that deficits cannot reduce transpiration by their effects upon the vapour pressure at the leaf cell surfaces alone, but must also cause an increase in the resistance to flow of water through the leaf cell protoplasts.

The results of previous investigations in this series are therefore confirmed.

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# Further Studies on Transport in the Cotton Plant.

## III. Concerning the Independence of Solute Movement in the Phloem.<sup>1</sup>

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With Plate I and nine Figures in the Text.

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<sup>1</sup> Paper No. 12 from the Physiological Department of the Cotton Research Station, Trinidad.



## I. INTRODUCTION.

IT seems clear from previous work that the bulk of the material exported from the foliage of cotton must travel via the phloem. Rapid longitudinal movement is restricted to the phloem region of the bark (22, 24). Elongated parenchyma cells run parallel with the sieve-tubes in the phloem of the cotton plant and comprise about half the total volume (cf. 25). Unless these cells differ very markedly from the other parenchyma cells of the bark they cannot, however, contribute appreciably to longitudinal movement, for transport of sugars in the cortical parenchyma is extremely slow and the rate of transport by ray parenchyma from bark to wood is apparently only of the same order as diffusion in water (24). Schumacher's (31) important work shows that a similar argument applies to the export of nitrogen from darkened leaves of *Pelargonium zonale*. He has further shown that eosin, which is apparently without effect on the phloem parenchyma, but which causes callus formation in the sieve-tubes, completely stops phloem transport. In the present paper it is noted, also, that on ringing the stem the phloem parenchyma of the petiole becomes filled with starch to such an extent that the path available for transport is enormously reduced. In spite of this restriction the export of nitrogen and certain other materials from the leaves was not appreciably affected. It is also noteworthy that parenchyma is absent from the phloem of Monocotyledons (3) and not universally present even in the Dicotyledons (14), while companion cells are not always continuously connected, and in conifers are absent (3). It seems certain from all the evidence available that rapid longitudinal transport through the phloem must be almost entirely restricted to the sieve-tubes.

The most complete theory of phloem transport is that of Münch (27), which postulates a mass flow of solution under a hydrostatic pressure gradient from regions of synthesis, or hydrolysis of reserves to regions of growth or condensation. The sieve-pores are assumed to be *open* and to allow of a rapid movement of sap, while movement of course must be mainly through the vacuole and not the cytoplasm. The energy required for this flow consists of the work involved in forming the solution of mobile materials in the parenchyma of the source and in condensing or otherwise transforming these materials in the parenchyma of the sink. There are, however, a number of serious objections to this simple and rather attractive mechanism.

(1) *The turgor gradient between the leaf parenchyma and the sieve-tubes* is the exact opposite of that required by the theory. The osmotic pressure of the leaf sap of cotton may average 13 atmospheres and that of the whole bark just below the foliage 12 atmospheres. In the sieve-tubes the osmotic pressure must be (cf. 24) two or three times as great as in the bark as a whole, while the *saugkraft* will be much less than that in the leaf. The

turgor of the leaf parenchyma must consequently be very much less than that of the sieve-tubes. Again Dixon (13) finds for sap exuding from the sieve-tubes of the main axis of the ash osmotic pressures of 27 to 35 atmospheres, while his values for the leaf sap are only from 15 to 20 atmospheres.

In an earlier paper on cotton (24) it was suggested that export of carbohydrate from leaf parenchyma to the sieve-tubes might take the form of diffusion of reducing-sugars, these being then condensed to sucrose, so building up a transport head. Metabolic energy would, of course, be required for this synthesis. Further work by Phillis and Mason (28) indicates that this simple explanation cannot be maintained and that a direct polar concentration of sucrose, from low concentrations in the parenchyma to high concentrations in the sieve-tubes, may be involved. In this case also metabolic energy would be required but, as in other cases of secretory activity by the cell, we are as yet quite ignorant of the mechanism by which the concentration is effected.

It seems, however, quite clear that the original theory of Münch is untenable and that mass flow of solution can be postulated only for the sieve-tube system itself, the energy required being supplied in the osmotic work of secreting material against a gradient into the sieve-tubes at the source. It should be noted that this modification of the Münch theory would meet the objection of Curtis and Scofield (11) that transport, presumably via the phloem, from storage organs to growing regions normally takes place against a gradient of osmotic pressure. The gradient of osmotic and turgor pressures *in the sieve-tube system* might, however, be in the right direction, material being secreted by the companion cells into the sieve-tubes of the source and removed, possibly even against a concentration gradient, from the sieve-tubes of the growing regions. Examination of the sieve-tube concentrations in these cases would be of very great interest.

(2) So far as the bark is concerned the existence of well-marked gradients of osmotic pressure in the normal direction of movement is established for cotton (26); while Dixon (13) has observed in *Fraxinus excelsior* gradients ranging from 2 to 9 atmospheres per metre in the case of sap exuded from the sieve-tubes. In view of the low values, usually well below 1 atmosphere per metre, calculated by Huber (18) for the vertical *saugkraft* gradients, it is clear that the turgor gradient cannot be very much below the total osmotic pressure gradient.

The difficulty, however, is the *resistance offered by the sieve-plates to mass flow*. Crafts (4, 5) has in fact rejected flow through the sieve-plates on this ground and makes the fantastic suggestion that the walls will offer less resistance to flow. The fallacy in his calculations of wall resistance has already been exposed by Steward and Priestley (34). Also the rates he observes for entry of dye solutions under pressure into bark (0.1 to 0.3 cm. per minute for a gradient of 1 atmosphere over only 3 cm.) show that for

normal flow pressure gradients far higher than those he has rejected for the sieve-plates would be required. He appears to be equally wrong in his conclusion that the *dimensions* of the sieve-pores exclude mass flow during exudation. He *calculates* that the initial exudation rate of 4.05 cm. per minute from the sieve-tubes of *Cucurbita Pepo* would require a pressure gradient of 20 atmospheres per metre and concludes that a pressure gradient of this magnitude cannot be accepted since the maximum osmotic pressure of the sieve-tube sap does not reach 20 atmospheres. But at the moment of cutting, and for a short time after, the pressure *gradient* must be many atmospheres *per centimetre*, i.e. greatly in excess of what is required. For normal transport in the intact plant the mass flow theory requires rates through the sieve-tubes of 0.3 to 2 cm. per minute (Münch 27). These rates, on the basis of Crafts's measurements for cucurbit sieve-plates would require pressure gradients of 1.5 to 10 atmospheres per metre, i.e. gradients that are of the same order as those actually observed in trees and in cotton (cf. p. 25). It would appear that the dimensions of the sieve-pores, except perhaps in the fine leaf veins of Dicotyledons (15) and in Conifers (17, 35) are not in conflict with the mass flow theory *provided the pores are normally open*.

Schmidt (33), Schumacher (32), and Crafts (6), however, conclude from microscopic observations that the pores are occupied by cytoplasm, in which case enormous pressure gradients would be required to force through solution. Before, however, any appreciable rate was attained under such pressures it seems likely that the protoplasm would be dislodged and the pore burst open. It is suggested that this may be the explanation of the exudation of sieve-tube sap observed when the sieve-tubes of certain plants are cut. It has long been known that a violent displacement of the protoplasmic contents takes place in sieve-tubes above and below a cut,<sup>1</sup> and an actual explosion through the pores is not unlikely. It appears to be quite unjustifiable, therefore, to argue from exudation rates on cutting to conditions in the intact plant; and a similar objection applies to measurements of rate of entry of fluid under pressure into sieve-tubes that have been emptied by exudation. Again, the sporadic nature of exudation very much diminishes its significance. In cotton no appreciable exudation has been detected, nor has it been possible to detect, under a pressure gradient of 0.75 atmospheres per 10 cm., any passage of water along the bark.

(3) The Münch theory involves movement of water along with the solute, and under certain circumstances this water must be liberated. Münch claims to have shown that flaps of bark prised away from the wood continue to grow and exude from the cambial surface considerable amounts of water. In similar experiments on cotton we have not been able to demonstrate any

<sup>1</sup> Schmidt's observations were for this reason made on material fixed *in situ* in the intact stem.

excretion of water though growth was vigorous. Weevers and Westenberg (39) have similarly been unable to confirm Münch's results.

(4) A further difficulty about the Münch theory is that it involves movement of all solutes in the same direction under the total turgor gradient. The nature of the materials imported into an organ must thus depend on the composition of the sap leaving the foliage and on the relative amounts of the different materials removed by the tissues around the *intervening* phloem track rather than on the specific growth requirements of the organ. Now the proportions of the different nutrients vary considerably from organ to organ and may vary also very considerably during the growth of each organ (e.g. fruits). Adjustments could be made only by the liberation of the nutrients present in excess into the xylem, so that a *return* flow of tracheal sap along the xylem would seem to be involved in the case of all organs except those whose growth requirements happen to coincide exactly with the composition of the sieve-tube sap in the axes leading to them. This would make the uptake by fruits of those nutrients which, like calcium, seem to be transported only in the xylem, extremely difficult.

Very recently van den Honert (37) has suggested that changes in surface tension brought about by the solution of assimilates and their removal at some remote point may be the cause of a mass movement of solution, since any substance causing a lowering of surface tension causes a recession of the surface layer from that point. The interface between vacuole and cytoplasm was suggested as the probable surface in which movement takes place. The vacuoles, however, of neighbouring sieve-tubes are not continuous through the sieve-plates, and this would restrict the movement to a single sieve-tube. Even if the vacuoles were continuous it is very doubtful whether an adequate force could be developed by the diminution of surface tension. The actual value of the force available cannot be computed, since we cannot estimate the part played by cohesion in determining movement.

The Münch theory of a unidirectional mass flow through the sieve-tube system regards this system as a series of inter-connected channels playing no part in transport except to restrict the stream to definite boundaries. There is, however, some evidence that transport is dependent on the activities of living cells in the transport path. The work of Curtis suggests that curtailment of the supply of oxygen to the petiole restricts the export of foodstuffs through the petiole (10). Moreover, Schumacher (32) has recently concluded from his experiments that the cytoplasm and not the vacuole is the channel of transport in the sieve-tube. Although in cotton we have so far found nitrogen and carbohydrate moving in the same direction along the phloem (i.e. in all cases away from the foliage to non-foliar regions) there are reasons for thinking that the two materials may move at independent rates, and in an earlier paper (25) we suggested that a critical test of the Münch theory would be the demonstration of movement of nitrogen and

carbohydrate in opposite directions along the phloem. The experimental work in the present paper was designed to test further this general possibility of independent movement of materials.

Before describing that work it is important to consider what kind of phloem mechanism would allow of the independent spread of the different materials. The streaming theory of De Vries (12) and of Curtis (10) would clearly satisfy the requirements, provided sufficiently high rates of mixing within each sieve-tube element and between neighbouring elements can be attained. Streaming must be rapid enough to produce an acceleration of diffusive spread up to a rate about 20,000 times as great as diffusion in water. It was pointed out in an earlier paper (25) that if exchange of solutes between successive sieve-tubes takes place by diffusion across the wall at the same rate as through water the maximum net acceleration attainable in cotton by streaming *within* the cell would be of the order of 1,000 at most. We should require therefore rapid streaming in and out of the pores of the sieve-plates. So far, however, no streaming has ever been observed in mature sieve-tubes, though rates of the order of a few centimetres per hour are fairly common in the young sieve-tubes and the phloem parenchyma. This is not an insuperable objection, for the operation of cutting the phloem must, as Curtis (10) emphasizes, disturb the mature sieve-tubes more than other cells. Huber (19) was, however, unable to detect any streaming in intact sieve-tubes. A more serious objection is the high rate that would be required to produce the observed acceleration of diffusive spread.

An approximate calculation can be made as follows:—In cotton the apparent diffusion constant<sup>1</sup> (cf. 24) is 0.07 (gm. cm.<sup>2</sup> sec. for a gradient of 1 gm. per c.c. per 1 cm.). There are about 20 sieve-tube units per cm. so that, on the assumption that streaming causes practically instantaneous mixing within each unit, each will, under unit gradient, differ from the next in series by a concentration of 0.05 gm. per c.c. If, now, mixing takes place by upward streaming from one unit to the next through half the sieve-pores and downward streaming through the other half, the net amount passing along under unit gradient will be  $\frac{1}{2}R \times X \times 0.05$  gm. per square centimetre per second, where  $R$  is the linear rate through the pores and  $X$  is the fraction of the sieve-plate occupied by pores. This amount must be equal to 0.07 gm. per cm.<sup>2</sup> per second, hence  $R \times X = 0.07 \div (\frac{1}{2} \times 0.05)$  cm. second = 2.8 cm. second. Thus taking a maximum estimate of pore area as  $\frac{1}{2}$  the total cross-

<sup>1</sup> This apparent diffusion constant has been calculated from the observed rate of transport per sq. cm. of 'sieve tube groups' in the phloem, and the estimated vertical concentration gradient in this tissue complex. About half the total area is made up of phloem parenchyma cells, the remainder being sieve-tubes and companion cells. The rate of transport per sq. cm. of actual sieve-tube would therefore be more than twice as great as that used in the calculations. As, however, the vertical concentration gradient may, like the transport, be restricted mainly to the actual sieve-tube track, it seems unjustifiable on this ground to increase our estimate of the apparent diffusion constant. This figure is therefore retained as an estimate of the order of acceleration required.

section we require rates of 5.6 cm. second or 336 cm. per minute.<sup>1</sup> Streaming at this rate seems quite impossible and, moreover, would require a tremendous rate of expenditure of energy.<sup>2</sup>

*The theory of sieve-tube transport by protoplasmic streaming would seem therefore to be as untenable as the theory of a directed mass flow.* If our conclusions as to the resistance offered by the sieve-plates are valid there can be normally no appreciable mass flow or mixing of fluid between successive sieve-tube elements and solute movement must be taking place in a relatively stationary medium.<sup>3</sup>

If phloem transport were polar the observed high rates of solute movement might be attained by polar concentration across each sieve-plate in the series from source to sink, like the polar concentration across the membranes between the mesophyll and the sieve-tubes of the fine veins. Transport along the phloem of the main-axis is, however, not polar. It is easily reversed, and is accompanied by reversal of the gradient of sugars, and in all probability by the gradient of mobile nitrogen. Moreover, variations in rate of downward movement of carbohydrate are definitely associated with variations in the downward gradient of sugars (24), and although the nitrogen (21, 22) relations are less simple, it appears that normal downward movement of nitrogen is associated with a gradient in the same direction of residual nitrogen—a fraction which on other grounds seems to be the mobile form.

What seems to be required therefore is a mechanism in the sieve tube system which enormously accelerates diffusive spread. Mason and Maskell (24) suggested the possibility of the existence 'of some special organization of capillary structure, which enormously reduces the resistance to diffusion'. If further work confirms Curtis's conclusion that curtailment of oxygen supply to the petiole checks transport, it seems unlikely that this suggestion can be entertained.<sup>4</sup> The acceleration of diffusion, like the polar concentration into the leaf sieve-tubes, will probably require expenditure of metabolic energy. In the case of polar concentration the energy required for the

<sup>1</sup> This is about 400 times the rate calculated from Crafts's figures for unidirectional flow.

<sup>2</sup> The energy required for this streaming through the plate may be roughly calculated as follows. With pores  $1\ \mu$  diameter and plate thickness  $5\ \mu$  ( $1/100$ th of the total sieve-tube length) flow of water at 336 cm. per minute through the pores would require a pressure gradient of 180 atmospheres per cm. across the plate, i.e. 180 atmospheres per metre along the whole system. The expenditure per 1 cm. cube of sieve-tube track will be  $1.8 \times 10^6$  ergs or  $4.39 \times 10^{-2}$  cals. Since 168 cm. are traversed in 1 minute, the expenditure in 24 hours will be  $4.39 \times 10^{-2} \times 168 \times 60 \times 24$  cals. = 1,060 cals. Since 1 gm. sucrose yields on combustion about 4,000 cals. this involves the respiration of at least 0.25 gm. of sucrose per 1 c.c. of sieve-tube sap per day, i.e. a 25 per cent. solution of sucrose in the sieve-tube sap would be completely exhausted in one day.

<sup>3</sup> Further evidence in the same direction is given by the bast glomeruli (23) of the Dioscoreaceae, for these consist of masses of compact parenchyma cells which interrupt the sieve-tubes at every node and would necessarily offer an enormous resistance to movement of solution.

<sup>4</sup> Unless metabolic energy is required to maintain the 'special organization'.

actual osmotic work can be calculated, since the departure from equilibrium is known. In default of any knowledge of the mechanism, we do not know, however, whether there is in addition an expenditure of energy in moving materials across the intervening membranes at the required speed. In the case of acceleration along the sieve-tube system no calculation of the necessary expenditure of energy seems possible; for we are concerned, not with departure from solute equilibrium, but with speeding up the rate at which solute equilibrium is reached.<sup>1</sup>

The facts could of course be interpreted by postulating unidirectional acceleration of spread across the membrane between the mesophyll and the sieve-tubes of the bundle-ends and anastomoses, and acceleration in all directions in the sieve-tubes. In spite of the difficulty of picturing the mechanism involved, some such theory, involving movement through stationary cytoplasm, becomes inevitable if mass flow through the sieve-pores is impossible and if materials move independently along the sieve-tube track according to their respective gradients.

## II. MOVEMENT THROUGH THE PETIOLE (EXPERIMENT 1).

### (A) *Introduction.*

In an earlier paper (24) an account was given of an attempt to reverse the normal direction of sugar transport through the petiole. The procedure adopted was as follows. In one group of plants about 30 per cent. of the leaves on each plant were darkened with paper bags. Another group with all the leaves darkened served as a control. All the plants were then ringed below the foliage region. It was thought that in the group having 70 per cent. of illuminated leaves, the sugar concentration in the bark of the stem adjacent to the darkened leaves might rise to a level at which backward movement through the petiole would take place. The carbohydrate content of the darkened leaves was estimated at intervals of six hours throughout the following day and night.

In the leaves of the wholly darkened plants the carbohydrate content diminished, but in the darkened leaves of the illuminated plants it increased slightly in time, and became, therefore, much greater than in the controls. Since some carbohydrate must have been lost by respiration, the increase observed was very suggestive of import. But as the actual increase in time was not statistically significant ( $P = \text{about } 0.07$ ) it was concluded that decisive evidence of import had not been obtained. In the absence of a quantitative estimate of respiration loss one could not exclude the theory that the observed effect was due solely to diminished rate of export.

<sup>1</sup> The formation of 1 litre of molar sucrose from a solution 20,000 times as dilute would require about 5,000 cals., an amount that would be supplied by complete combustion of just over 1 gm. of sucrose. This calculation gives us, however, no clue to the *rate* of energy expenditure needed to accelerate diffusion 20,000 times.

Either interpretation, i.e. import or diminished export, would, of course, be in harmony with the general picture of carbohydrate movement along the phloem as determined by sugar gradients. In view, however, of the marked response shown by sugar concentration in the bark, the small response of the leaf was surprising, and indicated that the gradient between mesophyll and phloem must possess special features. It was noted that the increase in sugar concentration in the bark was mainly due to sucrose, the percentage increase in reducing-sugars being small and almost exactly the same as in the leaf. The small import by the leaf could therefore be explained on the theory that carbohydrate interchange between mesophyll and phloem was restricted to reducing-sugars and was determined by their gradient only.

This theory afforded, at the same time, a possible explanation of the normal export of sugar from the leaf, since, although the sucrose concentration is very much greater in the phloem, there was evidence that the concentration of reducing-sugars in the phloem (sieve-tube groups) might be less than in the leaf.

More recently a special study of the gradient relations between leaf and phloem (28) has indicated that this simple theory cannot be maintained and that polar concentration of sucrose may be involved.

Whatever the mechanism of carbohydrate interchange between mesophyll and phloem it does appear, however, that a small amount of import by the mature darkened leaf can be observed. Import from a basal illuminated region of a cotton leaf into an apical shaded portion can readily be demonstrated in the following way. The whole leaf is darkened until the iodine test indicates absence of starch, and then a cardboard strip is clipped across the apical part of the leaf. If the stem is now ringed below the foliage region and the leaf illuminated, starch appears in the veins, and later in the lamina of the shaded part. The covered region remains starch-free *unless the plants are ringed*. The type of response is shown in Pl. I, Fig. 1. It will be seen that starch is present in the veins of the covered part, and that it is absent from the veins of the region exposed. The sequence of events on illumination is usually as follows. Starch first of all appears in the lamina of the exposed part; after about a couple of days it is seen in the veins of the covered region, and *later* appears in the veins of the exposed and the lamina of the covered part. Now the concentration of sugar in the exposed veins must be greater than in the covered veins, so that starch is formed at a lower concentration in the covered than in the exposed veins. The explanation appears to be that the humidity (cf. 1) is higher under the cardboard, but our experiments on this matter are not yet conclusive. The failure of starch to appear in the covered region of *unringed* plants (cf. 16, 30) long after it has appeared in the exposed region, is due, no doubt, to the fact that in the exposed region the concentration



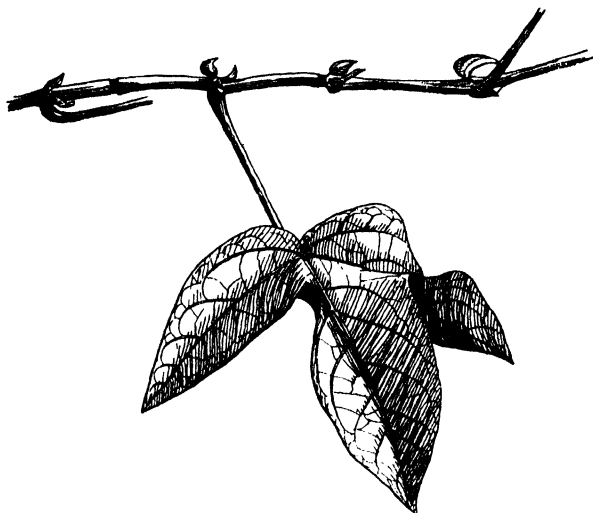
in the vicinity of the chloroplast is higher than the concentration in the rest of the cell, and this again must be greater than the concentration in the cells of the covered region. On ringing, however, the concentration in the cells of the shaded part rises until it reaches the threshold concentration for starch formation. The question of starch formation in the leaf would seem to depend largely on the ease with which sugar escapes from the chloroplast, for this will determine the concentration at the chloroplast.

We next attempted to make the whole leaf import sugar. The leaves were destarched by darkening, and the stems were then ringed. This time, however, only about 5 per cent. of the leaves were kept darkened. After about three days, starch appeared in the veins (cf. Pl. I, Fig. 2), and later began to spread into the lamina. In unringed plants no starch was found. A reversal of the normal direction of sugar transport through the petiole is thus indicated. Now as a net loss of nitrogen, and therefore export, can readily be determined on darkening the leaves of some plants (cf. 31), it occurred to us that it might be possible to bring about a simultaneous movement of nitrogen and carbohydrates in opposite directions through the petiole.

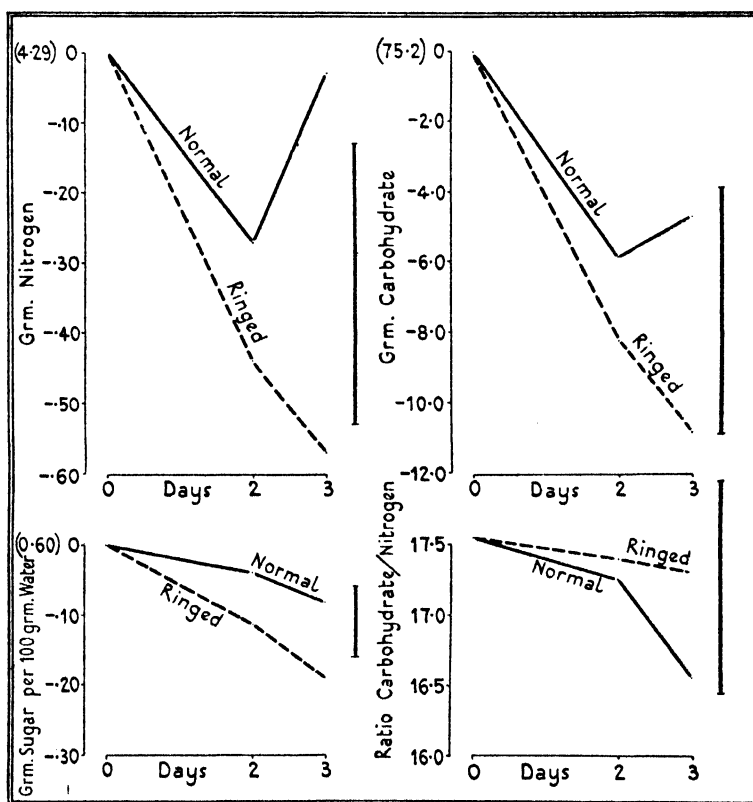
#### (B) *Procedure.*

When mature cotton leaves are kept darkened for a couple of days, which is the time required (cf. p. 31) for the appearance of starch in the leaves, the tendency to undergo abscission is greatly increased. It would have been an advantage, therefore, if the changes in carbohydrate and nitrogen content could have been followed by sampling successively portions of individual leaves. Unfortunately, in the time which the experiment requires, both the fresh weight and also the residual dry weight of darkened leaves diminish, so that there is no constant basis for calculating the content. We have thus been compelled to use a large number of leaves and estimate the content per one hundred leaves. A certain amount of shedding occurred, and it is uncertain to what extent it may have been selective.

Leaves on the fruiting-branches were used. Grading was done on the basis of length and breadth. About 5 per cent. of the leaves on the plants were covered with paper bags with a black inner and a white outer surface. All plants were ringed between the foliage region and the root, and on alternate plants two additional rings were made on the fruiting-branch on each side of a darkened leaf as shown in Text-fig. 1. These plants constituted the Ringed group. In the Normal group the only ring was that isolating the foliage region from the root. There were four samples at each collection, and there were twenty-five leaves per sample. The sequence of events was as follows:



TEXT-FIG. 1. Showing method of ringing fruiting-branch in Ringed group.



TEXT-FIG. 2. Showing change in weight of nitrogen and weight of carbohydrate per 100 leaves. Changes in total sugar concentration and the ratio  $\frac{\text{carbohydrate}}{\text{nitrogen}}$  are shown below. The values in brackets are the initial values. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

*Time-table.*

March 23, 1931, 6 a.m. Leaves covered with bags.

March 24, 1931, 8 a.m. Initial collection of Normal leaves and ringing of all stems and of fruiting-branches of Ringed group.

March 26, 1931, 1 p.m. First collection of Ringed and Normal leaves.

March 27, 1931, 1 p.m. Second collection of Ringed and Normal leaves.

*(C) Results.*

The results are shown in Text-fig. 2, and are, of course, expressed on the sample basis. The carbohydrate values represent the dry weight less 5.7 times the weight of nitrogen. It will be seen that there was a considerable loss both of nitrogen and of carbohydrates from the Ringed leaves. By the third day of the experiment the losses in this group were, for nitrogen 13 per cent., and for carbohydrate 14 per cent. The loss of carbohydrate may be in large part due to respiration; loss of nitrogen is presumably due, in the main, to export into that part of the fruiting-branch lying between the two rings. Import of nitrogen into the leaf via the xylem has probably been checked as a result of the drop in transpiration due to covering, while proteolysis has continued, and may even have increased as a result of the lowered sugar concentration (cf. 29). The leaves of the Normal group, which are in phloem connexion with the whole of the illuminated foliage region, show a much smaller loss, both of carbohydrate and of nitrogen, while, after the second day, both carbohydrate and nitrogen increase. These increases are, however, relatively small, being +5.5 per cent. of the initial value for nitrogen and +1.5 per cent. of the initial value for carbohydrate. For statistical significance an increase of about 9 per cent. would be required in both cases, so that the data give no definite evidence of import of either carbohydrate or nitrogen. A small import of carbohydrate seems probable, for some of the veins of the Normal group showed starch on the second and third days, while none was present in the Ringed group. Whether or not there was, after a preliminary loss, import of carbohydrates and nitrogen by the Normal leaves, there can be no doubt that the net export from the Normal leaves is definitely less than that from the Ringed leaves. On the concentration gradient theory of transport this diminished export would be due to the increased concentration both of carbohydrates and of nitrogen in the phloem of the fruiting-branch adjacent to the darkened leaves of the Normal group. On Münch's mass flow theory it would be due to the increased total osmotic pressure in the phloem. The facts are thus in accord with either theory.

On the basis of Schumacher's results for nitrogen loss from darkened leaves (31) it was anticipated that in the Normal group nitrogen might continue to be exported, while the export of carbohydrate was diminished or even reversed. This expectation has not been realized. As the ratios of carbohydrate to nitrogen in the two groups show (Text-fig. 2), nitrogen export has been diminished rather more strongly than carbohydrate export. The differences between the two groups are not, however, statistically significant.

In conclusion, it would appear that, in cotton, export both of carbohydrate and of nitrogen from darkened leaves may be reduced by maintaining phloem connexion with illuminated foliage, but that actual import into darkened leaves is difficult for both materials. It has not yet been possible in cotton therefore to determine whether carbohydrate and nitrogen can move in opposite directions along the petiolar phloem.

### III. MOVEMENT OF MINERAL NITROGEN UP THE STEM (EXPERIMENT 2).

#### (A) *Introduction.*

It was pointed out in an earlier paper (25) that, if Curtis's (7, 8) identification of the phloem as the normal path for upward transport of nitrogen and of inorganic salts absorbed by the roots were correct, we should have a case of completely independent movement of materials along the phloem track, for salts travelling upwards would move in opposition to the downward transport of carbohydrates. Critical examination of Curtis's results shows, however (cf. 25), that upward movement via the xylem and re-export from the foliage region provides an equally good explanation for the experiments in which xylem continuity was maintained, while in the experiments (9) in which the wood was severed, although there was some evidence of upward movement of substances other than carbohydrates, there was no evidence of downward movement of carbohydrates.

For the cotton plant the evidence seems clear (25) that if there is normally any upward movement of nitrogen or other materials via the bark as well as via the wood it cannot be detected, being completely overshadowed by the upward movement via the wood and the re-export and downward movement via the phloem. This applies to plants in an active vegetative condition and growing in the open. It was realized that these results did not exclude the participation of the phloem in upward transport, and it was planned to test other experimental conditions. In the first place it seemed desirable to test whether, under conditions of diminished transpiration, nitrogen supplied to the roots could be made to travel up the phloem. For this purpose plants low in nitrogen content were defoliated and placed in a humid atmosphere. Nitrogen was then supplied to the

roots, and the upward movement of nitrogen taking place via the xylem only and via the bark only was compared with that taking place along the intact stem.

(B) *Procedure.*

The plants, which were grown in a sand culture rather deficient in nitrogen, were, at the commencement of the experiment, about 30 cm. high. Thirty-five were chosen and graded into five grades according to height. One plant, chosen at random from each grade, was assigned to each of seven comparable samples. Of these samples two constituted the Normal group, two the Cut-bark group, two the Cut-wood group, and one was used for the initial collection. In the Cut-bark group a ring of bark was removed from the middle of the first internode at the base of the stem. In the Cut-wood group the bark of the first internode was cut longitudinally on opposite sides of the stem, a piece of wood about 3 mm. long removed, and the exposed surface covered with warm paraffin. In making collections for analysis the plants were separated into two regions, the part above the first foliar node, which is termed Top, and the part below the cotyledonary node, which is termed Root. The internode between the cotyledonary node and the first foliar node was fixed for microscopic examination.

The five plants in each sample at each collection were analysed individually so that an estimate of the sampling error per collection could be obtained.

The sequence of events was as follows:

*Time-table.*

January 19, 1931, 3 p.m. Cultures leached and supplied with solution rich in nitrogen, plants defoliated, and bell-jars containing wet cotton wool placed over plants.

January 20, 1931, 8 a.m. Initial collection. Bell-jars removed one by one, plants sprayed with water and bark or wood removed as described above, and bell-jars replaced.

January 24, 1931, 8 a.m. First collection of Normal, Cut-bark, and Cut-wood groups.

January 28, 1931, 8 a.m. Second collection of the three groups.

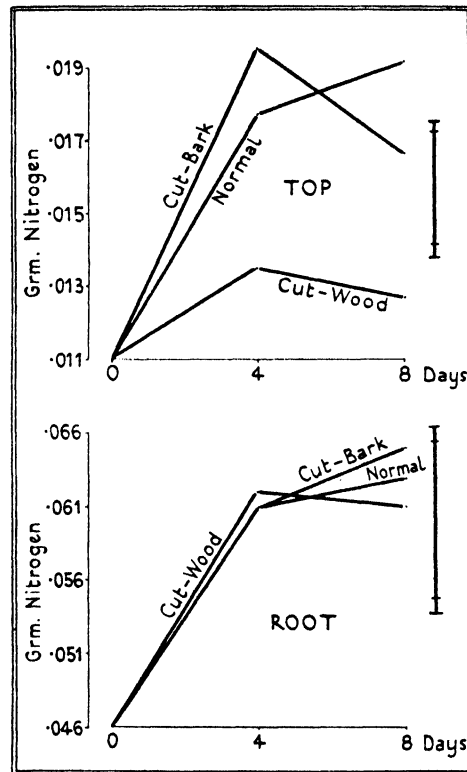
The bell-jars were removed daily, and the plants sprayed with water.

(C) *Results.*

The mean weight of nitrogen per plant in Tops and in Roots for each collection is shown in Text-fig. 3. The vertical lines on the right of the graph show what differences between any two collections would be signifi-

cant ( $P = 0.05$ ). Values for dry weight are not given, since this showed no significant variation either in time or as between different groups.

It will be seen that the Roots show a rapid increase in nitrogen content during the first period, after which there is little change, and that all three



TEXT-FIG. 3. Showing weight of nitrogen per plant in Top and Roots of the three groups. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

groups behave alike. In the Tops, on the other hand, while the Normal and Cut-bark groups show rapid and fully significant increases, the Cut-wood group shows a very much smaller increment which is not statistically significant. Thus, even when transpiration is drastically reduced by defoliation and high humidity, the xylem only is able to transport upwards as much nitrogen as is transported upwards in the intact plant. The phloem, on the other hand, seems to take little, if any, part in upward transport even under conditions of diminished transpiration rate. The statistically insignificant result for the Cut-wood group does not of course exclude the possibility that there was really some upward movement via the phloem. Moreover, examination of the phloem at the place where the wood was cut

away showed that there had been some damage. It was noticed also that sap exuded from the cut surface of the wood in this group, so that the smaller *total* uptake of nitrogen recorded for the Cut-wood group might have been due to loss of nitrogen by exudation. Thus the failure to establish upward movement via the phloem only might conceivably be due, partly to the slight damage to the phloem and partly to the fact that the competing channel, the wood, was not completely blocked but could still transport nitrogen away from the root. In the intact plant, however, where the xylem is not blocked, that tissue appears to be capable of transporting upwards all the nitrogen that passes to the tops while the phloem plays little, if any, part.

It is worthy of note that the nitrogen passing up the xylem in the Normal and Cut-bark groups must have been present in the tracheal sap in unusually high concentration. In spite of the humid atmosphere there was probably some transpiration, for, though the tops of the Cut-wood group remained turgid, they lost, taking the *average of the two collections*, 0.29 gm. water (9.5 per cent. of the initial weight per plant, 3.06 gm.). The average gain by the Normal and Cut-bark groups over the same period was 1.21 and 1.01 gm., so that, assuming their transpiration loss to be no more than twice the value for the Cut-wood group, we have, as maximum estimates for the water moving upwards, 1.79 and 1.59 gm. The amounts of nitrogen moving upwards were 7.4 and 7.0 mg., so that the concentrations in the tracheae were of the order of 0.4 gm. nitrogen per 100 c.c., or, as sodium nitrate, about 2.5 per cent. (cf. 2, 25).

#### IV. MOVEMENT OF STORAGE NITROGEN UP THE STEM.

##### (A) *Introduction.*

We saw in the preceding section that even under conditions of low transpiration rate it was not possible to establish upward movement via the phloem of nitrogen absorbed by the roots. The possibility remained, however, that upward movement via the phloem of storage nitrogen might be more easily demonstrated. Cotton, in common with many other plants, absorbs soil nutrients, during the early stages of growth, in amounts that are apparently in excess of immediate requirements. This excess is stored in the older regions at the base of the plant. If nutrient supply is now restricted the apical regions should draw on the reserves stored in the base. The experiment to be described was designed to test whether this upward movement occurred in the phloem, since, if so, it would be taking place in opposition to a downward movement of carbohydrates towards the basal regions and the roots.

(B) *Procedure.*

The plants were grown in sand culture under a glass roof, and were supplied with a full nutrient solution.<sup>1</sup> Sowing took place on March 19, 1930. Vegetative branches were not allowed to develop, and all flower-buds were removed. The plants were thus maintained in a vegetative condition throughout the course of the experiment. After one node had been produced on each fruiting-branch further growth was stopped by removal of the terminal bud. The tops of the plants thus consisted (cf. Text-fig. 4) of main-axis, leaves on main-axis, and a single leaf on each fruiting-branch.

The sand was leached with rain-water two days before the experiment began, and leaching continued throughout the course of the experiment. At this time the plants averaged 80 cm. high, and the mean number of fruiting-branches was twenty. On the basis of their heights the plants were divided into fourteen comparable samples of nine plants each. Of these, two samples were assigned to the initial collection, six to a Normal group, and six to a Ringed group. Each plant was subdivided, by means of wool tied around the stem, into four regions, namely (1) *Apical Upper*, comprising the four topmost fruiting-branches, (2) *Apical Lower*, comprising the next four fruiting-branches, (3) *Basal*, which extended from the Apical Lower region to sand level, and (4) *Roots* (see Text-fig. 4). The plants for the initial collection and for the Normal group had no further treatment. In the Ringed group quarter-inch rings of bark were removed between the apical and basal regions and between the basal and the root regions (see Text-fig. 4). The rings were, as usual, vaselined. The initial collection, two samples, was then made, and subsequently three collections, each of two samples, were made from Normal and from Ringed groups. The results will be given as mean values, per sample of nine plants, at each collection.

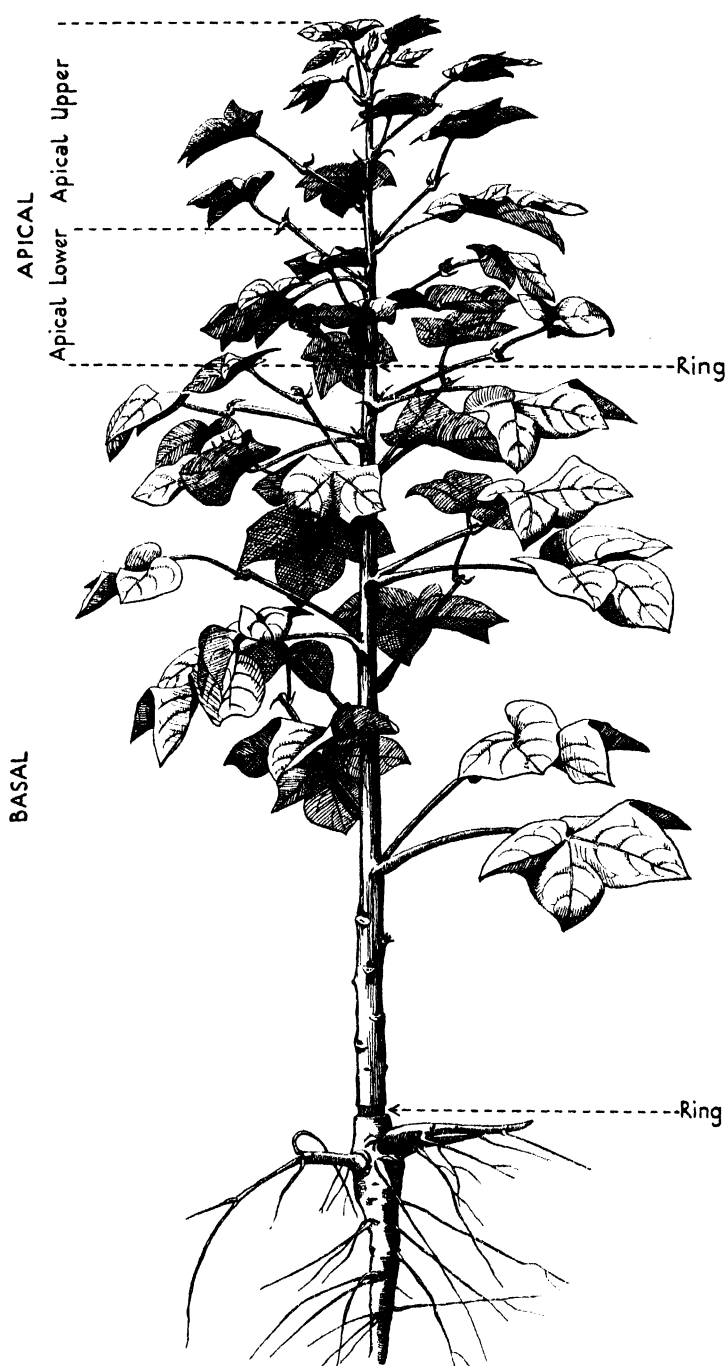
*Time-table.*

- May 31. Leaching with rain-water.
- June 2. Ringing and initial collection.
- June 6. First collection of Ringed and Normal plants.
- June 12. Second collection of Ringed and Normal plants.
- June 26. Third collection of Ringed and Normal plants.

In making the collections the plants were first subdivided into the four marked regions, the small portion of stem in the region of the ring being rejected in both groups. The root region consisted of all the roots that could be collected after washing on a millimetre sieve. The other regions

<sup>1</sup> The composition of the solution was as follows: p.p.m. N. 200; S. 131; Ca. 100; K. 94; Mg. 49; P. 48; Fe. 10; Al. 0.5; Cl. 158; Na. 45; Mn. 1.5; Zn. 1.0; B. 0.5.





TEXT-FIG. 4. Showing position of rings in Ringed group.

(stems, leaves, and petioles<sup>1</sup>) were handled separately and, except in the case of the apical-upper region, where the material was too immature, the bark and wood of the stem were separated.

Before presenting the results some comment must be made on the position chosen for the rings. It seemed likely from previous work (21, 22) that the bulk of the stored material would be in the basal region and that this would, in the Normal plant, move downwards to the roots and upward to the apical region. If this movement, both upwards and downwards, takes place mainly via the phloem, it should be checked by the two rings delimitating the basal region. The apical and root regions of the Normal plant should gain nitrogen at the expense of the basal region, while in the Ringed plant the basal region should not be able to export either to apex or to roots. The ring below the basal region enables us to test whether that region can export nitrogen upwards by direct leakage into the tracheae of that region.

Although, as the subsequent discussion will show, this arrangement of rings does not enable us to exclude certain other possibilities for upward transport in the Normal plant, it does give important information as to the route that may be taken.

In addition to information as to the movement of nitrogen, the scheme of ringing adopted should enable us to decide whether there was, during the experiment, downward movement of carbohydrates via the phloem out of the apical region. This is important, since we are testing whether this can take place at the same time as nitrogen is moving upwards via the phloem from basal to apical regions. Since the carbohydrate problem is rather simpler than the nitrogen problem it will be convenient to discuss the carbohydrate results first.

### (C) *Results.*

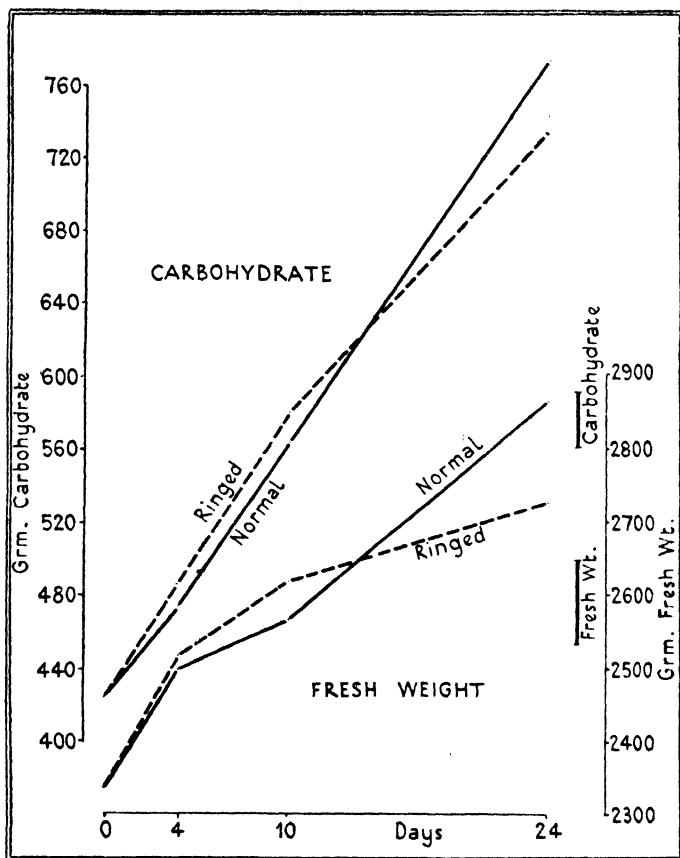
#### 1. *Carbohydrates.*

##### (a) *The whole plant.*

We have again used the dry weight less 5.7 times the weight of nitrogen as an estimate of the weight of carbohydrate material. Text-fig. 5 shows, for each collection, the mean fresh weight and total carbohydrate for the whole plant, including the roots. The vertical lines show what difference between any two collections would be statistically significant ( $P = 0.05$ ). Up to the time of the second collection both groups increased at about the same rate. The differences between Ringed and Normal groups at the first and second collections are not even partially significant ( $P$  is  $> 0.10$ ). By the time of the third collection, however, the Normal group shows a

<sup>1</sup> This category includes the first internode of the fruiting-branch.

fully significant excess over the Ringed. The total carbohydrate increment in the Ringed group from the initial collection to the third collection is 11 per cent. lower than that in the Normal group, while the increment in fresh weight is 27 per cent. lower. The excess shown by the Normal group is

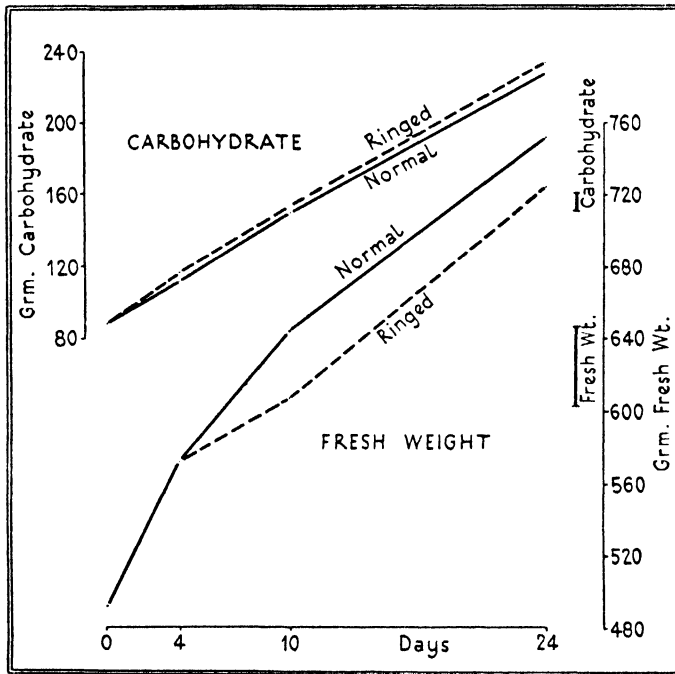


TEXT-FIG. 5. Fresh weight and weight of carbohydrate per sample for whole plant. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

probably due to the fact that the rings hindered the movement of nitrogen and other stored materials into the apical region, and so limited the development of new leaves. From the results for the first two collections it would seem that the rate of carbon assimilation by mature leaves was not depressed by the ringing. Possibly the rate was already depressed in both groups by the check to carbohydrate utilization for growth consequent on curtailment of nutrient supply. One might have expected, however, a somewhat greater depression in the Ringed group, for here the root region cannot import carbohydrate, and this, by accumulating in the leaves, might depress photosynthesis.

(b) *Apical region.*

The results for the whole apical region above the upper ring are shown in Text-fig. 6. The lower part of this region (apical-lower) was relatively mature at the commencement of the experiment, while the upper part



TEXT-FIG. 6. Fresh weight and weight of carbohydrate per sample for whole apical region. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

(apical-upper) consisted of tissues so young that bark and wood could not be separated. It will be seen that the weight of carbohydrate is greater in the Ringed than in the Normal group at all three collections. Although the individual differences are not statistically significant, the mean excess (4.90 gm.) of the Ringed group over the Normal would have occurred by chance in less than 5 per cent. of trials. (The  $P = 0.05$  level is 4.68 gm.) It seems, therefore, that carbohydrate has accumulated in the apical region of the Ringed group in excess of the Normal. This in itself suggests that, in the Normal group, carbohydrate has been moving downwards from the apical region towards the base. This conclusion is further strengthened by considering the foliar development in the two groups. The Normal group had at the end of the experiment 19 per cent. more leaves than the Ringed group, the average excess for the three collections being 10 per cent. Again, the fresh weight was greater in the Normal group (cf. Text-fig. 6). It seems reasonable therefore to conclude that the total carbon

assimilation by the apical region in the Normal group must have been definitely greater than in the Ringed. Thus the excess carbohydrate in the ringed apical region is in all probability a considerable under-estimate of the amount of carbohydrate that moved down from the apical region in the Normal group.

As would be expected, the excess of the Ringed over the Normal group appears only in the lower, more mature, part of the apical region. As Table I shows, the excess here is fully significant.

TABLE I.

*Mean Differences in gm. Carbohydrate between the Two Groups for Apical-upper and Apical-lower Regions.*

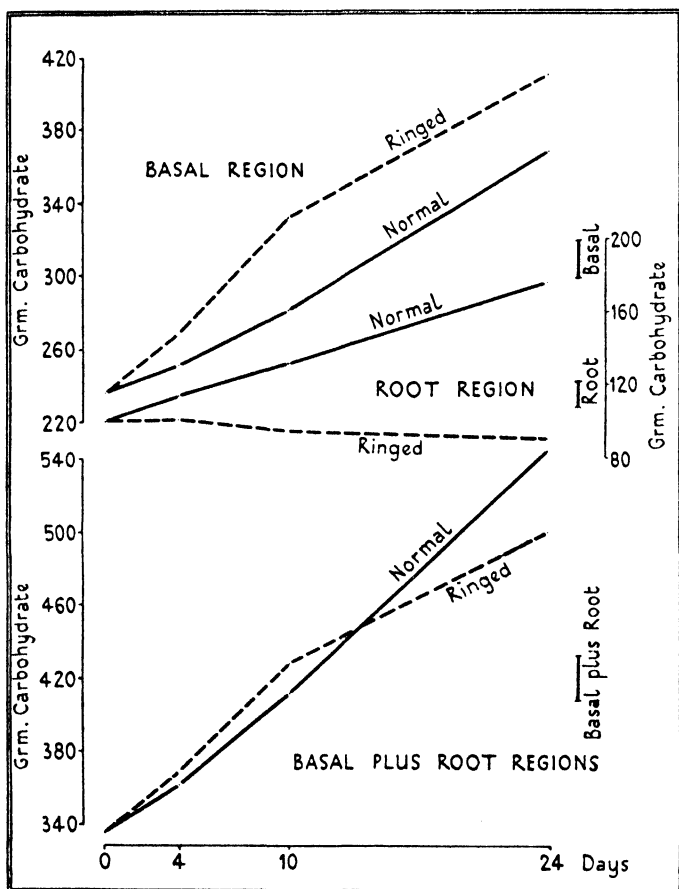
	Apical-upper.	Apical-lower.
Mean value for Normal	56.5	107.0
Mean difference = Ringed - Normal	-5.37	10.3
Significant difference $P = 0.05$	2.31	5.95

In the immature apical-upper region, on the other hand, the Normal is significantly in excess of the Ringed, though for the apical region as a whole the Ringed still shows the greater quantity. Thus while the ring, by preventing downward movement of carbohydrate, has led to accumulation of carbohydrate in the more mature tissues it has at the same time hindered development of the immature upper region. It is unlikely that this latter effect can be due to water strain, for at no time could signs of wilting be detected. It is almost certainly due to the fact that, as we shall show later, ringing checked the upward movement of stored nutrients to the apical region.

(c) *Basal and root regions.*

In the basal region (Text-fig. 7) the increase for the Ringed group is very definitely in excess of that for the Normal. In the root region the Ringed group shows a decline, while the Normal group shows a very definite increase. Clearly in the Normal plant there has been downward movement of carbohydrate via the phloem from basal to root regions. At the first two collections the amount held up in the basal region by the ring appears to be a little greater than the amount gained by the root region of the Normal plant. The excess at these two collections in favour of the Ringed group is not, however, significant. (See totals for basal + root regions in lower half of Fig. 7). By the time of the last collection, however, the amount held up in the basal region by the ring is quite definitely less than the amount gained by the root region of the Normal plants. No new

leaves had been formed in the basal region, and the mean fresh weight of leaves was, if anything, slightly in favour of the Ringed group. It seems, therefore, that the smaller carbohydrate content in basal + root regions of the Ringed group is in all probability due, not to diminished total assimilation in the basal region of this group, but to downward movement of



TEXT-FIG. 7. Weight of carbohydrate per sample for basal and root regions, and also for combined basal + root regions. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

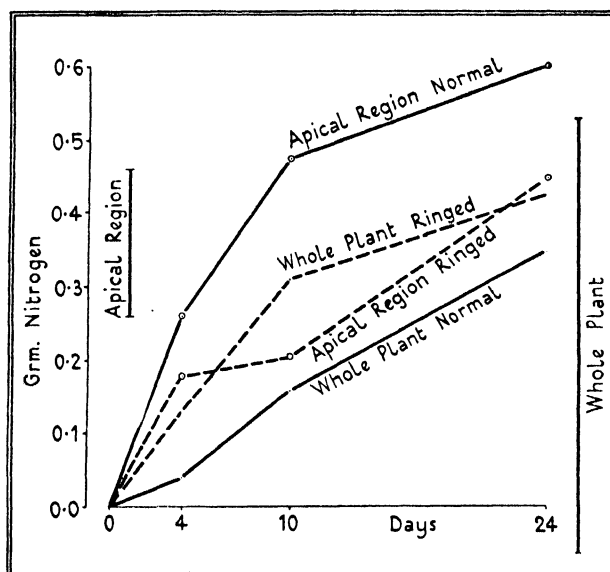
carbohydrates from the apical region in the Normal plant. In view of the fact that the results for the apical region lead to the same conclusion, downward movement of carbohydrates in the Normal plant, via the phloem, across the junction between the apical and basal regions may be regarded as established.

## 2. Nitrogen.

### (a) The whole plant.

The object of the experiment is to study the redistribution of the

nitrogen stored in the basal regions. It is important, therefore, to determine first whether any nitrogen has been taken up by the plant from the leached sand. The total nitrogen content at the initial collection was 7.087 gm. At the time of the third collection the amounts found in the



TEXT-FIG. 8. Increments in weight of nitrogen per sample for whole plant and for apical region. The initial values are: apical region, 2.695 gm.; whole plant, 7.087 gm. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

Normal and Ringed groups were 7.434 and 7.513 gm. respectively. The values for the first and second collections are intermediate. This suggests a very small uptake of nitrogen. Even for the lower level of statistical significance ( $P = 0.10$ ) we should, however, require an increase of 0.47 gm., whereas the maximum apparent increase is 0.426 gm. The quite regular increase in total nitrogen content of the whole plant in both groups (cf. Text-fig. 8) does, however, suggest that nitrogen was absorbed by the roots during the experiment. This may, nevertheless, be deceptive, for the whole set of values lies within the limits of sampling error. Thus, while an uptake of the order of 5 per cent. of the initial value cannot be excluded, there is no statistical evidence that even this small fraction has actually been taken up.

(b) *Apical region.*

Text-fig. 8 shows the results for the whole apical region and also for the whole plant. In the Normal group the increment in the apical region is fully significant from the start, and is, moreover, very much greater than

the apparent increase in the whole plant. The greater part, and possibly even the whole of the increment, must therefore have come from the storage reserves in the lower part of the plant.

In the Ringed group there is a fully significant increment in the apical region, but this is much less than the increment for the Normal group (the mean increment for the Ringed group is only 62 per cent. of that for the Normal). The difference between the two groups is fully significant.

Again, the increment in the apical region of the Ringed group is not very different from the apparent increment for the whole plant in this group, so that there is no evidence, for this group, of nitrogen stored in the base being transported upwards. The increase in the apical region of the Ringed group may well be entirely due to mineral nitrogen absorbed during the course of the experiment. This may seem unlikely as the basal region, having a greater fresh weight of leaves, and being separated from the roots by one ring only, might have been expected to show an equal or greater import of nitrogen from the transpiration current, and of this there is no sign. It must, however, be recollected that the young leaves are in vascular connexion with the young roots, and further that salt uptake may be very largely restricted to the young roots.

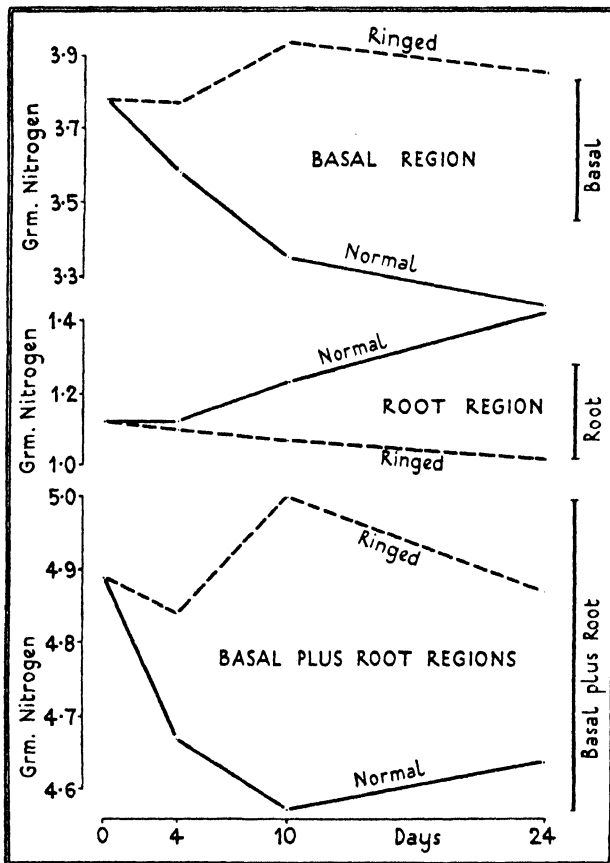
We have now to consider the significance of the difference between Normal and Ringed groups. Clearly ringing has considerably reduced the upward movement of nitrogen into the apical region. The most obvious explanation is that the excess in the Normal group represents storage nitrogen that has moved up via the phloem.

Since, however, considerable amounts of nitrogen ascended exclusively via the wood in the Ringed group, we must consider whether such movement might not account for the excess in the Normal group. As we have seen, the greater part of the nitrogen entering the apical region of the Normal plant represents nitrogen withdrawn from the basal regions. If upward movement takes place via the xylem only, then either (1) more nitrogen must be liberated into the tracheae of Normal plants, or (2) more of what is liberated must find its way into the apical region. It will be remembered that the apical region of the Normal plant developed more leaves and had at the end of the experiment a rather greater fresh weight than the corresponding region in the Ringed plant. Hence a greater proportion of the transpiration current might have passed to the apical region in this group. This explanation cannot, however, be accepted since the greater leaf area in the normal apex is undoubtedly the *result* rather than the cause of the increased increment of nitrogen and other stored materials. A plausible suggestion is that the ring between apical and basal regions diminished the fraction of the total transpiration current passing beyond the ring. On this assumption the same amount of storage nitrogen might be liberated into the tracheae of the basal regions and root, but in the



Ringed plant a larger proportion would be diverted again into the foliage of the basal region.

It will be convenient to discuss this possibility, and the alternative



TEXT-FIG. 9. Weight of nitrogen per sample for basal and root regions, and also for combined basal + root regions. Significant differences ( $P = 0.05$ ) are shown by the vertical lines.

possibility that more nitrogen was liberated into the tracheae in the Normal group, in connexion with the results for basal and root regions.

(c) *Basal and root regions.*

Text-fig. 9 gives the weights of nitrogen for the basal and root regions and for the combined basal + root regions in each group. In the basal region the Normal group shows a fully significant loss, while the Ringed group shows a slight apparent gain which is well within the sampling error. In the root region the Normal group shows a fully significant increase, while the Ringed group remains practically unchanged. Comparing the amounts present in basal + root regions, we note that while the Ringed group shows no real change the Normal group decreases. The mean

difference between Ringed and Normal groups is fully significant (mean difference = 0.275; significant difference (for  $P = 0.05$ ) = 0.243). It is clear, therefore, that export of stored nitrogen has taken place in the Normal plant from the basal region via the phloem to the roots, and also via the phloem or via the xylem, to the apical region. We have already concluded that the increase in the apical region of the Normal plant must be due to stored nitrogen, and the present results show that this stored nitrogen has come from the basal region.

On the assumption that this nitrogen has been liberated into the tracheae, and has moved up via the xylem, we have to explain why there

TABLE II.

*Initial Values and Percentage Increase or Decrease in the Tissues of the Basal Region, at the Time of the Last Collection.*

Nitrogen.	Bark.	Wood.	Petioles.	Leaves.	Total.
Initial values gm.	0.293	0.327	0.240	2.92	3.78
Percentage { Ringed	+210	+57	+55	-29	+2
change { Normal	+82	+10	+13	-29	-15
Carbohydrate.					
Initial values gm.	33.0	73.6	36.4	93.3	236
Percentage { Ringed	+160	+87	+70	+33	+74
change { Normal	+108	+82	+41	+22	+56

should be no apparent liberation of nitrogen into the tracheae in the Ringed group. We noted earlier that, owing to the presence of the upper ring, a smaller fraction of the nitrogen liberated into the tracheae of the regions below might pass to the foliage of the apical regions. On this view only a fraction of the total nitrogen liberated into the tracheae in the basal and root regions would escape upwards, the remainder being retained by the foliage of the basal region to be later re-exported to the stem.

So far as the basal region in this experiment is concerned this effect of the upper ring, if it exists, should, however, be offset by the greater accumulation of nitrogen in the stem tissues of the Ringed group, which would be expected to lead to an increased rate of liberation into the tracheae.

The results for the separate tissues of the basal region are shown in Table II. The loss of nitrogen from the leaves is much the same in both groups,<sup>1</sup> but while, in the Normal group, most of this exported nitrogen

<sup>1</sup> It was noticed that the phloem parenchyma of the petioles of the Ringed group was crammed with starch, while the sieve-tubes as usual appeared quite empty. That this reduction in area available for transport in the parenchyma cells should have so little effect on rate of nitrogen export from the leaf confirms Schumacher's conclusion that the phloem parenchyma can take little or no part in actual transport.

made its way to the root and apical regions, in the Ringed group it all accumulated in the bark and wood of the main axis and in the petioles.

That in spite of this accumulation of nitrogen in the main axis no detectable loss of nitrogen from the basal region should have occurred, argues very strongly against any appreciable liberation of nitrogen into the tracheae of this region in the Ringed group. And if none occurred in the Ringed group still less should any have occurred in the Normal group with its much smaller accumulation.

It would appear, then, that the export of nitrogen from the basal to the apical region in the Normal plant cannot have taken place by liberation of nitrogen into the tracheae of the basal region. This failure of nitrogen to leak into the tracheae of the stem recalls Curtis's (7) observation that carbohydrate is not exported via the wood from a region of stem between two rings (cf. also 38). There is, however, a further possibility, namely, that liberation of nitrogen into the tracheae can take place, at an appreciable rate, in the root region only. Mineral nitrogen absorbed by the roots of the cotton plant leaves the root via the xylem (cf. p. 38). The frequent occurrence of organic nitrogen in the tracheal sap (25) suggests that stored material also may normally be liberated into the tracheae of the root region. It is also of course possible that some of the mineral nitrogen is transformed during its journey across the living cells of the root (36). In an earlier paper by Mason and Maskell (25) attention was drawn to evidence suggesting that the export and downward movement from the foliage, via the phloem, of nitrogen and some other nutrients, might normally be taking place in amounts that were in excess of the growth or storage requirements of the lower part of the plant. If this is actually the case then the excess must at some point be liberated into the tracheae and ascend the plant again via the xylem. From the present results it would appear that, in the case of nitrogen at least, this liberation into the tracheae could only occur in the region of the root.

On this view the nitrogen exported from the basal to the apical region of the Normal plant has moved downwards via the phloem to the roots, has there been liberated into the tracheae, and has ascended to the apical region via the xylem. Against this view is the fact that no appreciable loss of nitrogen has occurred from the root region of the Ringed plants, in spite of the fact that for twenty-four days these have been supplied with rain-water only. To overcome this objection we have to make the further postulate that this liberation of nitrogen can proceed only while the root is in phloem connexion with the foliage.

If this is the correct interpretation of the results obtained, the failure of export from basal to apical region in the Ringed group is due, not to the ring between apical and basal regions, but to the ring which isolates the basal region from the root. Clearly for a full analysis of the situation

two other Ringed groups would be required, one having a single ring between basal and root regions and the other having a single ring between apical and basal regions. On the hypothesis we are considering the second or High Ring group should show export from basal to apical regions, while the first or Low Ring group should show no such export. On the theory of independent movement of materials via the phloem the results should be rather in the other direction.

Thus the present experiment, in which, owing to the large number of plants required for adequate sampling, only one type of ringing could be attempted, is only a step towards complete analysis of the situation. Undoubtedly the simplest explanation of the results obtained is that upward movement of stored nitrogen towards the apical region takes place via the phloem and, consequently, in opposition to the downward movement via the phloem of carbohydrates. The alternative, that this upward movement is taking place in another channel, the xylem, is not excluded, but is seen to involve the provisos that the necessary liberation of nitrogen into the tracheae is restricted to the root, and can only take place even there while phloem connexion with the foliage is maintained or while there are abundant stores of carbohydrate. The view that the nitrogen exported from the foliage region must travel downwards via the phloem with the carbohydrates is of course a necessary consequence of Münch's mass-flow theory of phloem transport, and since flow from the foliage region will be determined mainly by the sugar gradient there may well be, at times, an excess of nitrogen which is liberated into the tracheae.

### *3. The behaviour of phosphorus, potassium, and calcium.*

It was shown in an earlier paper (25) that, when a ring is made between the foliage region and the roots, phosphorus and potassium, like nitrogen, accumulate in the stem above the ring and diminish in the stem below the ring. It was argued that this indicated downward movement, via the phloem, of material that had travelled to the foliage via the xylem. This type of response was clearly established for nitrogen and for phosphorus in all experiments. In the case of potassium, although the response was quite definite in most cases, in one it was so small as to be within the limits of sampling error. For calcium, on the other hand, there was no evidence of re-export from the foliage or of movement via the phloem. It will be of interest, therefore, to examine the behaviour of these three elements in the present experiment.

The essential data, with the levels of statistical significance, are given in Table III. This shows, for each region and for the whole plant, the amount of each element (gm. per sample) at the initial collection and the increment in Normal and Ringed groups up to the time of the final collection.

(a) *Phosphorus.*

The whole plant shows a small gain in phosphorus, which is significant for the Normal group. The difference between Ringed and Normal groups is not significant. In both groups the gain by the apical region is greater than the gain by the whole plant, indicating that some mobilization and upward movement of stored material has taken place. In the Normal plant this must have come from the basal region which shows a partially significant decrease. In the Ringed plant the basal region shows no loss

TABLE III.

*Uptake and Redistribution of Phosphorus, Potassium and Calcium. Initial Values (gm. per sample) and Increments in Normal and Ringed Groups at the time of Final Collection.*

(a) Phosphorus.	Initial value.	Increment.		Significant difference.	
		Normal.	Ringed.	$P = 0.05.$	$P = 0.10$
Whole plant	1.282	+ 0.114	+ 0.057	0.106	0.084
Apical region	0.351	+ 0.136	+ 0.105	0.038	0.030
Basal region	0.650	- 0.059	+ 0.005	0.073	0.058
Root region	0.281	+ 0.027	- 0.053	0.058	0.047
Basal + root	0.931	- 0.032	- 0.048	0.077	0.061
(b) Potassium.					
Whole plant	4.172	+ 0.149	- 0.008	0.296	0.235
Apical region	1.558	+ 0.540	+ 0.482	0.145	0.115
Basal region	2.100	- 0.605	- 0.395	0.155	0.124
Root region	0.514	+ 0.215	- 0.096	0.121	0.096
Basal + root	2.614	- 0.390	- 0.491	0.171	0.137
(c) Calcium.					
Whole plant	7.22	+ 1.25	+ 1.07	0.59	0.47
Apical region	1.403	+ 0.768	+ 0.393	0.157	0.125
Basal region	5.206	+ 0.315	+ 0.693	0.58	0.46
Root region	0.608	+ 0.167	- 0.011	0.079	0.063
Basal + root	5.814	+ 0.482	+ 0.682	0.564	0.452

whatever, the mobilized phosphorus being apparently supplied by the root, which shows a partially significant decrease. The gain by the apical region is greater in the Normal plant than in the Ringed, the difference being partially significant. As in the case of nitrogen, this difference might be due either to material moving up direct via the phloem from basal to apical regions, or to material exported from the basal region via the phloem to the root, and from there moving up via the xylem. It will be noted that, like nitrogen, phosphorus is not liberated in appreciable quantities into the tracheae of the stem, for the basal region of the Ringed group shows no loss. As in the case of nitrogen, this failure to export from the ringed basal region is not due to failure of export, via the phloem, from the foliage, for the leaves lost over 30 per cent. of their initial phosphorus, while the stem tissues gained 84 per cent.

Thus in the upper part of the plant phosphorus shows a close resemblance in behaviour to nitrogen. In the ringed root, however, there is some evidence of leakage of phosphorus into the tracheae, while there was no evidence of leakage of nitrogen. The postulate that certain materials, moving down the phloem, which cannot be liberated into the tracheae of the stem may yet be liberated into the tracheae of the root has, therefore, some experimental support. There is, however, the difficulty that in the case of nitrogen this liberation, if it ever occurs, must depend on abundant carbohydrate supplies or phloem connexion with the foliage, while in the case of phosphorus it obtains under conditions of starvation. Further work is of course required before these apparent differences of behaviour can be regarded as definitely established. The phosphorus results do, however, make rather more plausible the view that export of stored material from the foliage to the apex may take place by downward movement via the phloem to the roots, liberation into the root tracheae and upward movement thence via the xylem. It should be noted, perhaps, that this view is in no way inconsistent with the theory that all materials that are mobile in the phloem move independently of one another. Direct upward movement via the phloem, in opposition to the downward movement of carbohydrate, may be taking place as well, the relative amounts of material passing upwards or downwards being determined by the gradients in these directions in the phloem of the main axis. The co-existence of two possible paths does, however, make the determination of the part played by each much more difficult.

(b) *Potassium.*

From the figures for the whole plant in Table III it is clear that uptake of potassium during the experiment has been negligibly small. The marked gain by the apical region is thus due to export of potassium from the regions below. As was the case for nitrogen and for phosphorus, the gain is greater in the Normal group, but the difference between the two groups is much smaller and is not significant. Clearly the double ring has done very little to hinder upward movement of stored potassium. The most interesting results, however, are those for the basal region, which shows a marked net loss in both groups. In the Ringed group this loss must have occurred by liberation direct into the tracheae of the stem. In the Normal group there is, in addition, the possibility of export via the phloem to the roots, if not to the apex as well. The results for the roots show a definite gain in the Normal group and a partially significant loss in the Ringed. In contrast to nitrogen and phosphorus, potassium appears to be liberated fairly readily into the tracheae at any point, so that ringing can do little to hinder the upward transport of stored potassium. We cannot, of course, exclude the possibility that, in the Normal plant some

of the potassium exported from the leaves of the basal region travelled direct from basal to apical regions via the phloem, but the figures suggest that the greater part must have ascended via the xylem. The export of potassium from the leaves of the basal region was far more marked than the export of nitrogen or phosphorus. Taking the initial values in leaves plus petioles as one hundred, export from the leaves was forty-seven for the Normal and forty-three for the Ringed plant. In the Normal plant thirty-six passed on via the phloem to the root and via the xylem (and phloem?) to the apical region, only eleven remaining in the stem of the basal region. In the Ringed plant twenty-four passed on via the xylem to the apex, nineteen remaining in the stem. These results confirm our earlier conclusions that the accumulation of potassium above a ring represents potassium moving downwards via the phloem from the foliage. At the same time they indicate that, even where vigorous downward movement of potassium is in progress, accumulation above a ring is likely to be seriously diminished by liberation of the potassium direct into the tracheae. This fact may explain why, as noted earlier (p. 51) the response of potassium to ringing is less consistent than that of nitrogen or phosphorus.

(c) *Calcium.*

In previous work on cotton (25) we noted that calcium differed from nitrogen, phosphorus, and potassium in that it showed no sign of re-export from the foliage or of mobility via the phloem. It will be seen from Table III that, whereas in the case of the other elements the increment for the apical region was consistently greater than that for the plant as a whole, the increment in calcium for the whole plant was much greater than that for the apical region. With the exception of the root region in the Ringed group, all regions in both groups show increases. There has been, apparently, no mobilization of stored calcium, the increments representing calcium absorbed by the roots and travelling up the xylem. The amount passing up into the 'tops' (apical and basal regions) is almost exactly the same in the Ringed as in the Normal group, the slightly greater total uptake by the Normal group being due entirely to the gain by its root region, which was of course increasing in dry weight. The distribution of the increment in the tops is, however, very different in the two groups. In the apical region the Normal group shows a definitely greater increase than the Ringed group, while in the basal region the increase is greater for the Ringed group, though the difference is not here significant. Since we are apparently dealing entirely with material absorbed by the roots, and moving up via the xylem, the greater uptake by the normal apex cannot be ascribed to material moving from basal to apical regions via the phloem. The results obtained may be due to the possibility, suggested earlier, that the presence of the upper ring diminishes the fraction of the total transpira-

tion current passing to the apical region, and increases the fraction passing to the foliage of the basal region. This type of ringing effect will, of course, only be important in the case of elements like calcium, which are re-exported very slowly, if at all, from the regions they reach via the transpiration current. In the case of other elements such as nitrogen, phosphorus, and potassium, re-export from the foliage via the phloem must considerably minimize the effect.

#### V. SUMMARY.

(1) The existing theories of phloem transport are discussed and it is argued that neither the Münch theory of mass flow nor the De Vries-Curtis theory of protoplasmic streaming gives a satisfactory explanation of the known facts. It is pointed out that in the cotton plant, movement of carbohydrates from the mesophyll into the sieve-tubes appears to involve concentration of sugars, energy for this work being provided by metabolism of the transition cells. It is urged that we must consider the probability of further metabolic energy being used to accelerate the spread along the sieve-tubes of the sugars and other phloem-mobile nutrients. No suggestion can at present be made as to the mechanism by which either the concentration by the transition cells or the acceleration of diffusive spread along the sieve-tubes is brought about, but it seems that the two phenomena may be in some way linked up.

(2) In order to test further the analogy with diffusion, which involves independent spread of different materials along the sieve-tube path, examination is made of certain special transport situations in which carbohydrates and nitrogen might be moving in opposite directions along the phloem track.

(3) *Movement along the petiole.* (a) Starch tests indicate a *slow* import of carbohydrate by mature darkened leaves that are in phloem connexion with illuminated leaves. Similar leaves isolated by ringing from the illuminated foliage show no import. (b) Darkened leaves isolated by ringing from illuminated foliage show a continued loss of total carbohydrates and of nitrogen. Similar leaves on unringed shoots show a much smaller initial loss followed by recovery. Total carbohydrates and nitrogen behave similarly, but in neither case is the gain statistically significant. Although, therefore, there are grounds for thinking that darkened cotton leaves can import carbohydrates via the phloem there is no evidence as yet that they can at the same time export nitrogen via the phloem.

(4) *Movement of mineral nitrogen up the stem.* Ringing experiments on the uptake and upward movement of mineral nitrogen in plants whose transpiration rate has been drastically reduced by defoliation and the maintenance of a humid atmosphere show that even under these conditions the xylem alone is able to transport upwards as much nitrogen as ascends via



the intact stem, while evidence of upward movement via the phloem only was not obtained. It is concluded that the phloem can take little, if any, share in the normal upward movement from the roots of mineral nitrogen.

(5) *Movement of storage nitrogen and other reserves up the stem.*

(a) It is shown that, when cotton plants, grown in full nutrient solution, are deprived of their nutrient supply, nitrogen and other nutrients are exported from the foliage of the basal region and move *upward* to the apical region. The data show that there is at the same time *downward* movement of carbohydrate via the phloem from apical to basal regions and roots. This export of nitrogen from the basal region is entirely prevented by ringing the stem above and below the basal region, a result which suggests that in the normal plant the mobilized nitrogen which reaches the apical region is moving up via the phloem in opposition to the downward movement of carbohydrates.

(b) It is shown, however, that an alternative theory, of ascent of mobilized nitrogen entirely via the xylem cannot be excluded, for the mobilized nitrogen might have all passed via the phloem to the roots, been liberated there into the xylem, and so ascended to the apical region. This explanation will fit the facts observed if we accept the proviso that this liberation of nitrogen into the tracheae is restricted to the root region and takes place there only while there is phloem connexion with the foliage.

(c) The behaviour of phosphorus is similar to that of nitrogen, and is susceptible of the same alternative explanations, with the exception that liberation of phosphorus into the tracheae can apparently occur in a root region isolated from the foliage.

(d) In the case of potassium, ringing has little effect on upward movement of reserves from basal to apical regions, though it stops export to the roots. It would seem that, unlike nitrogen and phosphorus, potassium exported from the foliage via the phloem is fairly easily liberated into the tracheae of the stem. It is concluded that most if not all of the upward movement of stored potassium can occur via the xylem.

(e) There is no evidence of remobilization of calcium.

(6) It is concluded that the upward movement of stored nitrogen may be an example of upward movement via the phloem in opposition to the downward movement of carbohydrates, but that until more is known as to the conditions determining liberation of stored nitrogen into the tracheae the alternative of upward movement entirely via the xylem cannot be excluded.

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## EXPLANATION OF PLATE I.

Illustrating the paper by Drs. T. G. Mason, E. J. Maskell, and E. Phillis on 'Further Studies on Transport in the Cotton Plant. III. Concerning the Independence of Solute Movement in the Phloem.'

Fig. 1. Showing distribution of starch. On left, exposed area (L), and on right, shaded area (D). Leaf treated with iodine. Photographed by transmitted light.

Fig. 2. Showing import of carbohydrates through petiole. On left, half leaf before import ; on right, half leaf after import. Both half leaves treated with iodine. Photographed by transmitted light.





# The Effect of Potassium Chloride on the Diurnal Changes of the Carbohydrates of the Potato Leaf.

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With four Figures in the Text.

## INTRODUCTION.

MASKELL (8) and James (7) have investigated the effect of manuring with potassic fertilizers on the properties and functions of the leaves of potatoes grown in the field. By staining the leaves with iodine, and comparing the colour developed with a standard scale of colour tones, Maskell compared the rate of starch production and disappearance in leaves from plants receiving no potash, and plants receiving potassium sulphate, potassium chloride or 'potash manure salts' (a mixture of potassium and sodium chlorides). He showed that the rate of starch production was increased by potassium sulphate, but not by the chloride treatments, and found evidence that the rate of starch removal was similarly affected. This was confirmed by James.

It is known from the work of Briggs (2) and Gregory and Richards (6) that a deficiency of potassium salts depresses the rate of photosynthesis, and it might be argued that the increased rate of starch formation observed in leaves of plants treated with potassium sulphate was due to an increased concentration of sugars produced by photosynthesis. James, however, adduces evidence to show that this is not the only effect, but that in addition the enzyme system controlling the condensation of sugars to starch is activated by the potassium salt. Using the inverse argument, the fact that potassium chloride had no effect on the rate of starch formation and removal does not necessarily indicate that the rate of photosynthesis was unaffected. It has been well established by field experimentation that application of potassium chloride tends to increase the yield of potato tubers, though to a less extent than an equivalent amount of potassium sulphate, and it is therefore probable that the photosynthetic efficiency of the plant is increased by the application of chloride.

The starch content of the leaf gives only a partial view of the changes consequent upon photosynthesis, and it is clear that a study of the changes

taking place in other carbohydrates is essential for a more complete knowledge of the effects of potassium salts. The method of estimation of starch employed by Maskell and James was not strictly quantitative, and it is possible that changes were produced by the chloride, but that the method used was not sufficiently sensitive to detect them. In view of these facts it was decided to investigate the effect of potassium chloride on photosynthesis and translocation, by determining the diurnal carbohydrate changes in the leaves by direct analysis. It was realized that it would have been advantageous to include in the experiment a comparison of the effect of potassium chloride and sulphate, and possibly also sodium chloride and sulphate, so as to discriminate between the cation and anion effects, but it was not found possible to do this on account of the labour involved.

The diurnal changes in the carbohydrates of the potato leaf and petioles have been investigated by Davis and Sawyer (3). The object of their investigation was primarily biochemical, to determine what carbohydrates were present in the leaf. From the physiological point of view they were mainly concerned with the problem of the 'first sugar of photosynthesis'. They concluded from this work, and from a similar investigation on mangold leaves, that sucrose is the first sugar of photosynthesis. The evidence from the changes in the leaf which led them to this conclusion was that sucrose was present in the leaf in greater amount than hexoses, that sucrose showed a regular diurnal fluctuation while hexoses varied irregularly and to a much smaller extent than sucrose, and also that sucrose reached its maximum concentration before starch, suggesting that it was a precursor of starch formation. It is now realized that this type of investigation is unlikely to furnish conclusive evidence on the question of the first sugar of photosynthesis. The relative amounts of reactants accumulated at any time in a series of linked reactions depends not only on the position of the reactants in the chain, but also on the relative rates of the successive reactions.

Davis and Sawyer's analyses were carried out on single samples, taken at each sampling time, and it is therefore impossible to determine directly the magnitude of the errors involved. It appears that no steps, beyond a rejection of very old and very young leaves, were taken to ensure uniformity of age and physiological status of the material sampled on successive occasions. The lack of an estimate of error renders it impossible to test the significance of the small and irregular changes in hexoses. Davis and Sawyer thought that they were caused by conversion of hexose into, or its formation from starch, since hexose and starch showed some negative correlation, but it is possible that they were due to errors of sampling.

The work of Barton-Wright and M'Bain (1), who studied the diurnal changes of carbohydrates in the leaves of potatoes grown in pots, is open to the same criticism. Also these authors have analysed their results by

the method of partial correlation, and their interpretation of the results of the analysis is of doubtful validity. For example, the correlation coefficient tests the goodness of fit of a straight line to the relation between two variates, and since the variation of the carbohydrate fractions with time is undoubtedly not linear, the correlation coefficients between carbohydrate fractions and time are of little relevance. The particular part of the day covered by the sampling must determine whether a significant correlation is obtained or not. It has frequently been pointed out that the establishment of a significant correlation between two variates,  $x$  and  $y$ , provides no evidence on the question of whether variation in  $x$  is caused by variation in  $y$ , or vice versa. Nevertheless, Barton-Wright and M'Bain have assumed that the use of partial correlations and the magnitude of the regression coefficients enables them to determine, for example, whether in the process of photosynthesis sucrose is formed from hexose, or hexose from sucrose.

In view of these difficulties in the interpretation of previous work, the experiment here described was arranged so that an estimate of error could be made.

#### MATERIAL.

The material was obtained from five plots of the potato block of the Six-Course Rotation at Rothamsted, full details of which are given in the Rothamsted Annual Report, 1932 (11). The treatment applied to these plots was as follows:—

Treatment.	Fertilizers applied. Cwt. per acre.		
	K <sub>2</sub> O (as KCl).	N (as sulphate of ammonia).	P <sub>2</sub> O <sub>5</sub> (as super-phosphate).
0K	0	} 0.3	} 0.3
1K	0.25		
2K	0.5		
3K	0.75		
4K	1.0		

The fertilizers were applied on 11th April, 1933, and the 'seed' planted on 12th April. The variety was 'Ally'. The area of each plot was 1/40th acre, and there were approximately 450 plants per plot. The treatments were applied at random within the block, so that a valid estimate of error could be made. It should be noted that there was no replication of treatments in the experiment; the estimate of error was obtained from the residual variance after fitting a regression on quantity of KCl applied.

The samples taken all consisted of leaflets from the pair next the terminal leaflet of the fourth leaf from the top of the haulm, so that the material used was of uniform age.

#### *Basis of expression of results and method of sampling.*

The results of investigations of carbohydrate changes in plants are usually expressed as a percentage of the dry weight. Changes in



carbohydrate fractions expressed on this basis are not a true measure of the absolute changes in amount since the dry weight of the plant, or part of the plant studied, changes during the course of the day. Mason and Maskell (9) suggested the use of 'residual dry weight' (dry weight less total carbohydrate) as a basis of expression, assuming that the major fluctuations in dry weight are due to changes in carbohydrate. This solution of the difficulty is only approximate, and Mason and Maskell point out that the ideal solution is to express the carbohydrate fractions as weight per leaf. In general this is impracticable on account of the large samples which must be taken to determine mean leaf weight with sufficient accuracy, but in the potato a considerable economy of sampling can be effected by making use of the correlation which exists between the weights of opposite leaflets of the same leaf. A similar procedure has been suggested by Denny (5).

In a preliminary sampling the standard errors of the mean dry weight per leaflet were determined for the comparison of corresponding leaflets from different plants, and for the comparison between leaflets of the same pair. The standard error of the dry weight per leaflet, for the comparison of leaflets from different plants was 59.3 per cent., and for the comparison of leaflets of the same pair, 11.9 per cent. A high correlation exists between opposite leaflets, so that while a sample of 140 leaflets would be necessary to give a standard error of 1 per cent. for the comparison with the sample of opposite leaflets, a sample of 3,500 leaflets would be necessary to obtain the same accuracy for the comparison between samples taken from different plants.

The procedure adopted was to take a sample of leaflets at the beginning of a sampling period, and at the end of the period to sample the opposite leaflets. The difference in dry weight of the two samples provided a measure of the change in the dry weight per leaflet during the sampling period. By repeating this process for each successive period it was possible to determine at each sampling time the dry weight of 100 gm. of initial leaf dry weight (see Appendix, Table XV). Thus the results could be expressed as the changes taking place in a quantity of leaf whose dry weight was 100 gm. at the beginning of the experiment, and the difficulty of a changing basis of expression was avoided by actually measuring and correcting for the changes.

The sampling was carried out on 17th–18th July, 1933, at three-hourly intervals from 9 a.m. (G.M.T.) on the 17th to 9 a.m. on the 18th. A sample consisted of 30 leaflets, approximately 10 gm. fresh weight, each leaflet taken from a different plant. A leaflet was taken from every fifteenth plant on the plot, a fresh random starting-point being selected from amongst the first fifteen plants in the first row at each sampling time. A random sample distributed over the whole plot was thus obtained. The sampling programme was as follows: At 9 a.m. a sample was taken from each of the

five plots (sample A). The leaves from which the leaflets were taken were conspicuously marked with a label, so that they could easily be distinguished later. At noon the opposite leaflets to those sampled at 9 a.m. were taken (sample B), and also a fresh sample from different plants (sample A). This process was repeated at each sampling time, so that there were two series of samples, the A series and the B series, of which each B sample consisted of leaflets opposite to those of the A sample of the previous sampling time. The A series were used for carbohydrate estimation, while the comparison of the dry weight of sample A at time  $x$  and sample B at time  $x+1$  gave a measure of the change in dry weight which took place in the period between time  $x$  and time  $x+1$ .

The dry weights of the A samples were estimated after alcohol extraction, by drying the alcohol insoluble residue and evaporating an aliquot of the alcohol extract at  $100^{\circ}\text{C.}$ , while the dry weight of the B samples was estimated directly by oven drying at  $100^{\circ}\text{C.}$  A comparison of the mean dry weight as percentage of fresh weight of the A series and the B series over the whole experiment (excluding the A sample of the first sampling time, at which no B sample was taken) is given in Table I.

TABLE I.  
*Comparison of Methods of Dry Matter Determination.*

Mean of 40 determinations.		Dry weight as percentage of fresh weight.	
A samples.	Alcohol extraction.		
	Insoluble residue dried and aliquot of extract evaporated at $100^{\circ}\text{C.}$	18.595	
B samples.	Oven dried at $100^{\circ}\text{C.}$	18.536	
	Difference	0.059	S.E. $\pm 0.087$

The difference between the values obtained by the two methods was small and not significant, so that no disturbance was introduced by the use of two methods of dry matter determination.

The sampling was carried out by four people, and each sampling occupied from 40 minutes to an hour. The five plots were sampled in random order at the first time, and the same order was used in the later samplings. Two of the samples taken at 9 a.m. were lost. The data for this time have therefore been omitted, and the results which follow apply to the eight sampling times from noon on the 17th July to 9 a.m. on the 18th July.

*Weather conditions during sampling.*

During the morning of the 17th July until 11.30 a.m. there was little cloud and bright sunshine. From 11.30 a.m. onwards the sky was overcast

with thin cloud, except for short spells of sunshine from 2.55 p.m. to 3.10 p.m., and from 5.50 p.m. to 6 p.m. There was a shower of rain beginning at 10.40 p.m. and lasting until 12.45 a.m. on the morning of the 18th July, heavy at first, but later very light. The early morning of the 18th July was cloudy until 6.30 a.m., when there was a very short and light shower. Later the sky was clear and there was bright sunshine until the end of the sampling period. The total rainfall was 1.0 mm.

The air temperature during the period varied only between 60° and 67° F. From 9 a.m. to 1 p.m. on the 17th July the temperature rose steadily from 60° to 67°. From 1 p.m. until 3 p.m. there was a slight fall to 65°, followed by a rise to 66.5° at 6 p.m. The temperature then fell steadily to 60.5° at 1 a.m. on the morning of the 18th July, and remained between 60.5° and 60° until 7 a.m., when it began to rise again to 64° at 9 a.m.

#### METHODS BY ANALYSIS.

##### (a) *Drying and extraction.*

The samples were taken to a field laboratory and weighed fresh as rapidly as possible. The B samples were immediately placed in the drying oven. The A samples were rapidly cut up and dropped into boiling 95 per cent. alcohol in large boiling tubes, sufficient alcohol being used to give approximately 80 per cent. alcohol with the water in the leaf. The extraction was continued for about half an hour, and the tubes were removed, stoppered, and stored until analysed. The pieces of leaf tissue were filtered off on stainless steel gauze, dried at 60° C., ground and extracted for a further period of about 8 hours. The insoluble extract was dried and preserved for starch estimation. The alcohol extract was evaporated at 30–40° C. under reduced pressure, until the alcohol was all removed, and the residue was taken up in water, cleared with basic lead acetate and sodium phosphate, and made up to volume.

##### (b) *Estimation of carbohydrates.*

The carbohydrates estimated were reducing sugar, sucrose, and starch. Reducing sugars were estimated in the cleared solution by the Schaffer-Somogyi method (12). Sucrose was determined by measuring the increase in reduction after hydrolysis with 0.4 per cent. HCl at 70° C. for 15 minutes.

Starch was estimated by a modification of the von Fellenberg method described by Denny (4). 0.5 gm. of the dried alcohol insoluble residue was ground up very finely with a few drops of saturated CaCl<sub>2</sub> solution. The paste was washed into a boiling tube with 25 c.c. of the CaCl<sub>2</sub> solution, and heated on a boiling water-bath for 1 hour under a reflex condenser. The liquid was made up to 50 c.c. with distilled water, the volume of insoluble

material being assumed negligible. An aliquot of the extract, after filtering off the insoluble residue, was hydrolysed by the addition of HCl, to give a concentration of 2.5 per cent. HCl, followed by heating on a boiling water-bath for 2½ hours. The solution was made just alkaline with saturated Na<sub>2</sub>CO<sub>3</sub> solution, and the CaCO<sub>3</sub> precipitated was filtered off. The filtrate was neutralized with 0.5 per cent. acetic acid and made up to volume. The glucose formed by hydrolysis of the starch was then estimated by the Schaffer-Somogyi method. The NaCl remaining in the solution after precipitation of the CaCO<sub>3</sub> affected the reaction slightly, but this disturbance was overcome by carrying out the blank determinations with the Schaffer-Somogyi reagent on a NaCl solution of equivalent concentration instead of on distilled water.

In the discussion of the results, the sum of sucrose and reducing sugar is referred to as 'total sugar', and that of total sugar and starch as 'total carbohydrate'.

#### EXPERIMENTAL RESULTS.

##### (1) *Method of statistical analysis.*

The primary data, consisting of the fresh and dry weights of the A and B samples and the carbohydrate and water contents as percentage of dry matter of the A samples, are given in the Appendix, Tables IV to IX. In addition, analyses were made to determine the K<sub>2</sub>O and Cl content of the leaflets, and these are given in Table II, together with the mean leaflet weights.

The derivative data necessary for the investigation of the time changes are given in Tables X to XVIII. The tables present the variations which took place throughout the sampling period in the dry matter, carbohydrate fractions, residual dry matter, and water contained in a quantity of leaf whose dry matter was 100 gm. at noon on the 17th July. The forty determinations of each variate form a 5 × 8 table with 39 degrees of freedom. The method of statistical analysis, which has been used, is illustrated in the following Analysis of Variance of Starch per 100 gm. initial dry matter.

##### *Analysis of Variance. Starch per 100 gm. Initial Dry Matter.*

				Sum of squares.	Mean square.
Between plots	4 D.F.	Linear regression on			
		KCl applied	1 D.F.	0.058	0.058
		Error (a)	3 D.F.	24.509	8.170
Within plots	35 D.F.	Times	7 D.F.	373.162	53.309
		Interaction. Linear regression × Times	7 D.F.	9.052	1.293
		Error (b)	21 D.F.	17.903	0.853
Total	39 D.F.			424.684	

Similar analyses have been carried out on the other variates and the standard errors calculated. The significance of the variances due to regression, times, and interaction have been tested by means of the 'z' test.

For ease of presentation, corresponding fractions of the analyses of all the variates will be discussed together. The three fractions thus considered are:

(1) The linear regression of the means of all times on quantity of KCl applied, which provides information on the mean effect of KCl over the whole sampling period.

(2) The variation between sampling times of the means of all levels of KCl.

(3) The interactions of the linear regressions with time, which measure the extent to which the effect of KCl varied at different times.

Second and third order regression terms were also calculated, but none were significant, and the linear regressions give an adequate expression of the results.

(1) *Leaflet weight and content of  $K_2O$  and Cl.*

The mean fresh weight, dry weight, dry matter per cent., and the  $K_2O$  and Cl content of the selected leaflet for each plot are shown in Table II, and the linear regressions on quantity of KCl applied, with their standard errors, are given.

TABLE II.

*Effect of KCl on Leaflet Weight, D.M. per cent., and  $K_2O$  and Cl Content.*

	Units of KCl applied (1 unit = 0.25 cwt. $K_2O$ per acre)					Linear regression on KCl applied.
	0	1	2	3	4	
Mean fresh weight per leaflet, gm.	0.24	0.30	0.33	0.38	0.33	0.027 $\pm$ 0.011
Mean dry weight per leaflet, gm.	0.049	0.058	0.060	0.066	0.057	0.0025 $\pm$ 0.0019
Dry Matter %	20.4	19.3	18.5	17.5	17.2	-0.827 $\pm$ 0.026
$K_2O$ % of D.M.	1.33	1.67	2.13	2.96	3.38	0.539 $\pm$ 0.065
Cl % of D.M.	0.360	0.512	0.712	0.996	1.331	0.243 $\pm$ 0.022
$K_2O$ per 100 gm. water.	0.340	0.401	0.486	0.629	0.696	0.094 $\pm$ 0.0094
Cl per 100 gm. water.	0.091	0.123	0.162	0.212	0.273	0.045 $\pm$ 0.0034

The leaflet weights are the means of the 16 samples (480 leaflets) taken from each plot. Both fresh weight and dry weight showed some tendency to increase with increasing KCl supply, but the linear regression was not significant in either case, and we must therefore conclude that the weight of

## *Diurnal Changes of the Carbohydrates of the Potato Leaf. 67*

the leaflet was not affected by KCl supply. Dry matter as percentage of fresh weight showed a significant decrease with increasing KCl supply, a result previously found by James.

The determinations of potassium and chlorine were made on the B series of samples after oven drying. The samples from each plot for the first four sampling times and for the second four were bulked, and separate determinations were made on the two bulked samples, but the differences between the two sets of samples were not significant. The means are given in Table II. The amount of both  $K_2O$  and of Cl in the leaf, whether expressed as a percentage of dry matter or per 100 gm. of water in the leaf, increased significantly with increase in KCl supply. It is probable, therefore, that any effect on the carbohydrates of the leaf was brought about directly by an alteration in the concentration of potassium or chlorine in the leaf.

The ratio of the regression coefficient of  $K_2O$  on KCl supply to the regression coefficient of Cl is 2.2. If additional potassium and chlorine passed into the leaf in equivalent amounts, as a result of increasing the supply of potassium chloride, the ratio of the regression coefficients would be 1.3. It is clear, therefore, that relatively more potassium was absorbed or translocated to the leaf than chlorine.

### *(2) Changes in dry weight, carbohydrates, and water content.*

#### *(a) Mean effect of potassium chloride over the whole sampling period.*

The means of all times and all quantities of KCl and the linear regression coefficients of the means of all times on quantity of KCl applied are shown in Table III.

TABLE III.

	Mean of all times, and quantities of KCl. gm. per 100 gm. initial dry matter.	Linear regression coefficient on KCl applied. (Mean increase per 0.25 cwt. KCl per acre.)
Reducing sugar	1.88	- 0.103 ± 0.078
Sucrose	2.33	- 0.205 ± 0.046
Total sugar	4.21	- 0.307 ± 0.106
Starch	8.44	- 0.027 ± 0.320
Total carbohydrate	12.65	- 0.334 ± 0.416
Dry matter	94.8	- 0.72 ± 2.39
Residual dry matter	82.1	- 0.29 ± 1.69
Water	421	+ 17.5 ± 11.2

The mean concentrations of reducing sugar and, particularly, of starch were higher than those found by Davis and Sawyer, while that of sucrose was slightly lower.

The regression coefficients were all negative with the exception of that of water, but the only one which was significant was that of sucrose.

*(b) Time changes of the means of all levels of KCl.*

The time changes of the means of all levels of KCl are shown by the full lines in Figs. 1, 2, 3, and 4, and the magnitude of the significant difference is indicated by a vertical line on each graph, whose length is equal to

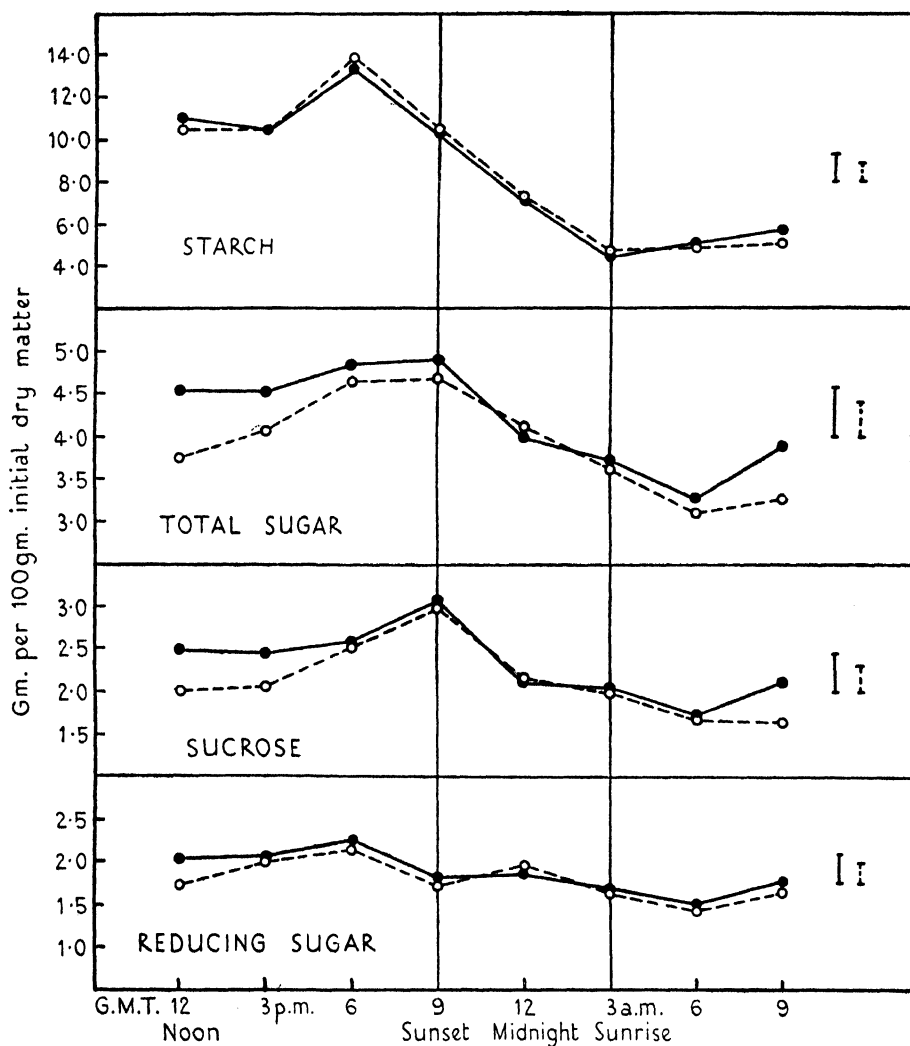


FIG. 1. For explanation see text.

$2.94 \times$  the standard error of the means ( $t = 2.08$  when  $n = 21$ ,  $P = 0.05$ ;  $\sqrt{2} t = 2.94$ ).

Significant changes were found in all the carbohydrate fractions (Fig. 1). From noon to 6 p.m. the changes in sugars were small and not significant, but starch increased significantly during this period. Between 6 p.m. and

9 p.m. both starch and reducing sugars decreased significantly, but sucrose showed a marked rise. The increase in sucrose and the decrease in reducing sugars were almost equal, so that the amount of total sugar remained constant during this time.

During the period of darkness from 9 p.m. to 3 a.m. starch and sucrose rapidly decreased, but there was no significant change in reducing sugar, though the curve shows some tendency to fall. Starch did not completely disappear from the leaf, but fell to about one-third of its maximum amount.

With daylight, starch began to increase, and the difference between the amounts present at 3 a.m. and 9 a.m. was significant. The sugars showed a continued tendency to fall until 6 a.m. The decrease in sucrose was greater than that in reducing sugar, but neither were significant, nor was the change in total sugar. From 6 a.m. to 9 a.m. both reducing sugar and sucrose increased, and though the changes in neither were significant alone, the increase in total sugar was significant.

Total carbohydrate (Fig. 2) reached its maximum value at 6 p.m. There was little change between noon and 3 p.m., indicating a low rate of photosynthesis, but from 3 p.m. to 6 p.m. there was a rapid and significant increase. From 6 p.m. to 3 a.m. total carbohydrate fell rapidly, and the fact that the rate of fall was almost as rapid between 6 p.m. and 9 p.m., as during the hours of darkness, indicates that the rate of assimilation was very low between 6 p.m. and 9 p.m. There was no significant change between 3 a.m. and 9 a.m., but the break in the curve at 3 a.m. and the subsequent tendency to rise, shows that the rate of photosynthesis was slightly more than sufficient to offset translocation loss. More than 50 per cent. of the total carbohydrate of the leaf was removed by translocation during the period from 6 p.m. to 3 a.m.

The largest change in dry matter (Fig. 2) took place during the hours of darkness. About 10 per cent. of the dry matter was removed by translocation between 9 p.m. and 3 a.m. The changes before 9 p.m. were small and not significant. There was no change between 3 a.m. and 6 a.m., nor was the increase between 6 a.m. and 9 a.m. sufficiently great to be significant.

Residual dry matter (dry matter—total carbohydrate) for the mean of all levels of KCl is also plotted in Fig. 2. None of the somewhat irregular fluctuations were significant.

Fig. 3 shows the water changes. The small and insignificant rise between 3 p.m. and 9 p.m. became more rapid after dark, and from 9 p.m. to 3 a.m. a significant rise took place. During this time the amount of water increased by 15 per cent. This increase may have been due in part to rain on the leaf surface, although the leaves were dried as completely as possible with filter paper. It was followed by a rapid and significant fall between 3 a.m. and 9 a.m.



(c) *Effect of KCl on the time changes.*

The dotted lines in Figs. 1, 2, 3, and 4 show the mean effect of one additional unit (0.25 cwt. per acre) of KCl, that is to say, the difference between a point on the full line, which shows the changes in the mean for

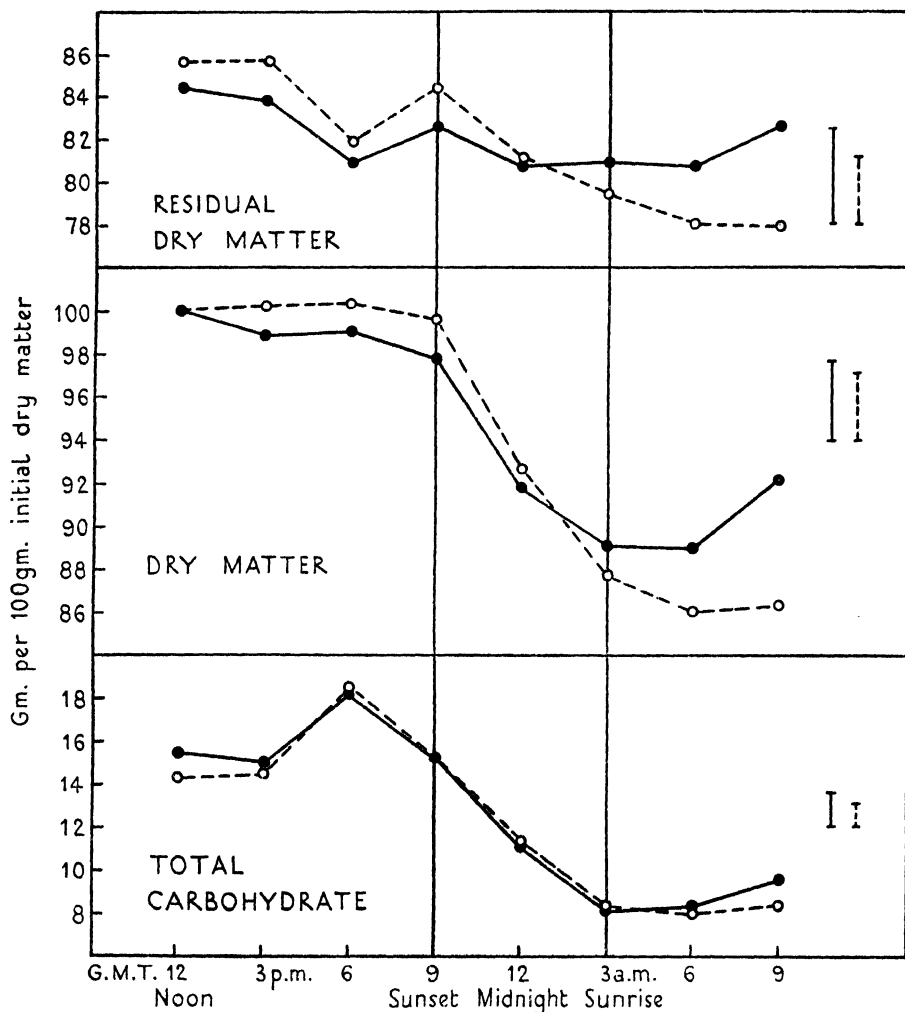


FIG. 2. For explanation see text.

all levels of KCl, and the point for the same time on the dotted line, represents the linear regression coefficient on quantity of KCl at that time. The small vertical dotted line is a measure of the significant difference between the linear regressions. It should be noted that the standard error from which this significant difference is derived is appropriate only for testing the significance of differences between regression coefficients. The

appropriate standard errors for testing whether the regression coefficient at a given time differs significantly from zero can be obtained from the residual variance of the values for the five plots at this time.

The interactions of the linear regression coefficients of starch and of

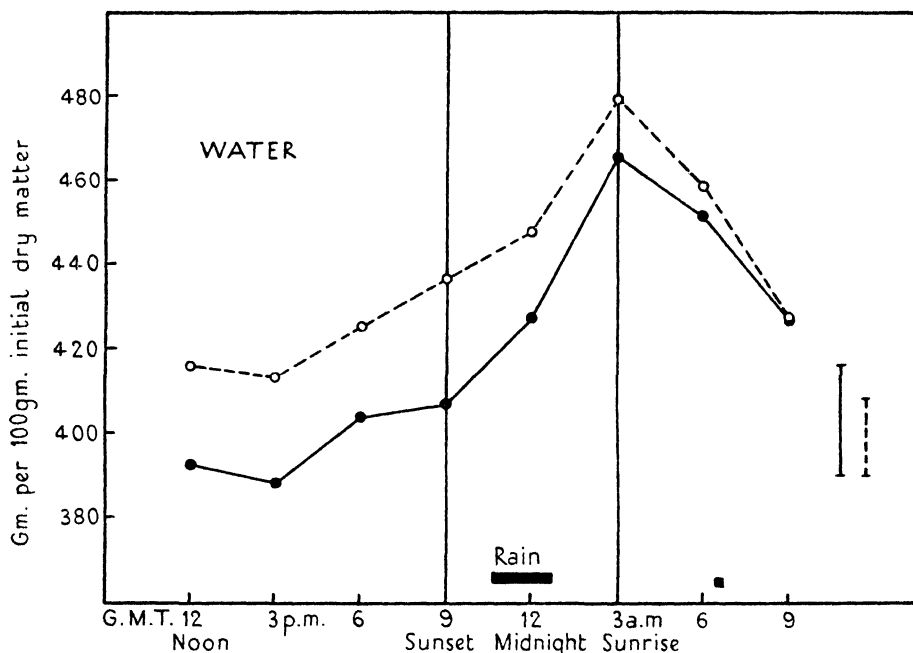


FIG. 3. For explanation see text.

reducing sugar content on KCl were not significant, and since the regressions of the means of all times were also insignificant, it must be concluded that application of KCl did not affect the concentration of either starch or reducing sugar in the leaf.

The regression coefficients of both starch and reducing sugar tended to have greater negative values at noon and 9 a.m. than at other times, and the coefficients for the noon sampling were just significant, when compared with the residual variance of the five values at this time. It should also be noted that the regression coefficient of starch was positive for all times between 6 p.m. and 3 a.m., and its magnitude steadily decreased from 6 p.m. to 3 a.m., becoming negative again at 6 a.m.

The results suggest, therefore, that starch and reducing sugar were slightly depressed during the morning by increased KCl supply, but the failure of the interaction terms (times  $\times$  regression) in the Analysis of Variance to reach the level of significance indicates that this may have been a chance effect.

It has already been shown that there was a significant regression of

the means of all times of the content of sucrose on KCl applied. The magnitude of the regression also showed highly significant variation with time, and the nature of this variation is obvious from Fig. 1. The regression coefficient had a high negative value at noon, 3 p.m., and 9 a.m., but fell to zero at the other sampling times. In other words, during the middle of the day, the amount of sucrose present in the leaf was depressed by increasing supply of KCl, but there was no effect in the evening, throughout the night, in the early morning.

The interactions of linear regression on KCl supply with time were significant for both total sugar (Fig. 1) and total carbohydrate (Fig. 2), and both showed the same cycle of changes. The highest negative coefficients were found at noon and 9 a.m. During the afternoon the negative coefficient steadily increased in magnitude, falling almost to zero during the hours of darkness, and beginning to increase again in the early morning. These changes were mainly due to the variation in sucrose.

The changes in the regression coefficient of dry matter on KCl supply (Fig. 2) were quite different from those of carbohydrates. Between noon and 9 p.m. the coefficients were positive, but during the hours of darkness they decreased rapidly, and from 3 a.m. onwards became increasingly negative. The difference between the positive coefficient at 9 p.m. and the negative coefficient at 3 a.m. was significant, so that during this time the rate of removal of dry matter from the leaf was increased by application of KCl. The changes in dry matter were not entirely attributable to carbohydrate changes. This is obvious when residual dry matter (Fig. 2) is examined.

The regression coefficient of residual dry matter on KCl supply showed the same change with time as that of dry matter. It decreased from positive values previous to 9 p.m., to zero at midnight, and increasingly negative values later. Table XVI shows that with low KCl supply residual dry matter tended to increase, and with high KCl supply to decrease over the period of the experiment, with the result that the mean of all levels of KCl did not change significantly. It is obvious that if residual dry matter had been used as the basis of expression of the carbohydrate fractions, the effects of varying KCl supply would have been distorted, though the time changes of the means of all levels of KCl would not have been much affected.

The regression coefficient of water per 100 gm. initial dry matter on KCl supply (Fig. 3) was large and positive at the beginning of the experiment, and steadily decreased to zero at the end. Application of KCl, therefore, reduced the rate of increase during the period of rising water content from noon to 3 a.m., and caused a more rapid decrease when the water content fell later. The effect can be attributed to a condition of equilibrium between water and dry matter in the leaf. Fig. 4 shows the

changes in water expressed on the basis of actual dry matter. Here the regression coefficient was positive and showed no significant variation; or in other words, application of KCl caused a constant increase in the weight of water associated with 100 gm. of dry matter of the leaf at any time.

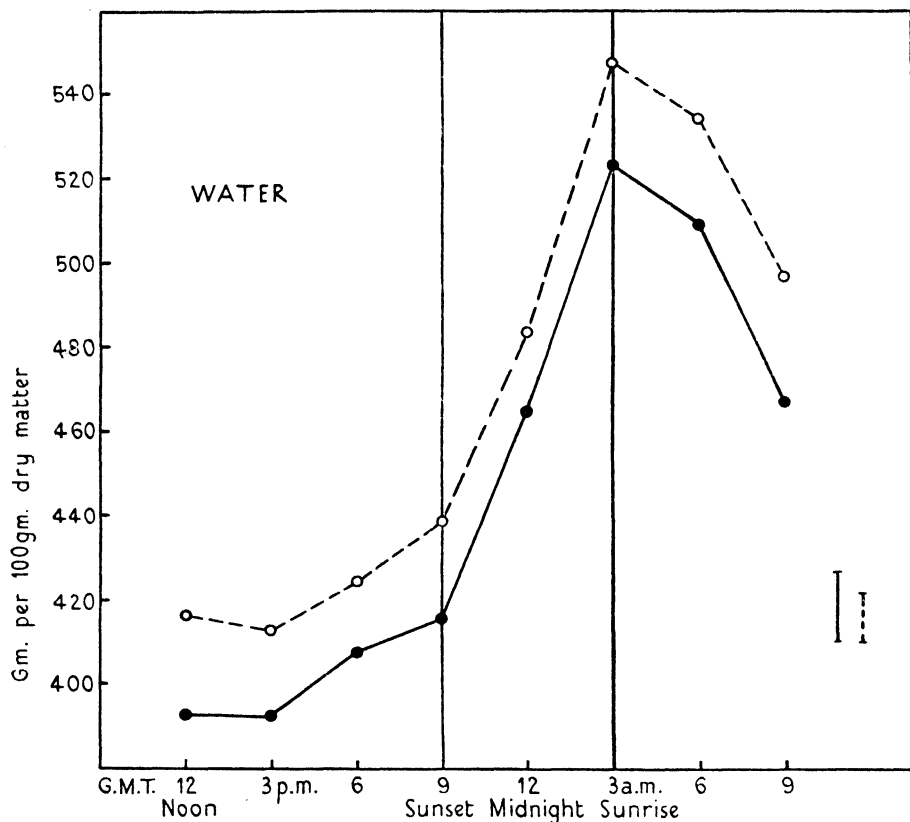


FIG. 4. For explanation see text.

The increased loss of dry matter from 100 gm. initial dry matter during the experiment, associated with increasing KCl supply, was accompanied by an equivalent loss of water, and thus the large positive effect of increased KCl supply on the water content per 100 gm. initial dry matter at the beginning of the experiment steadily diminished as the experiment progressed.

#### DISCUSSION.

(a) *Diurnal changes.* (Means of all levels of KCl.)

The changes with time may conveniently be considered in three phases. During the first phase, from noon to 6 p.m., the total carbohydrate in the leaf increased, so that the rate of photosynthesis was

greater than the rate of translocation. The increase in total carbohydrate was mainly due to production of starch, for both reducing sugars and sucrose changed very little. The net rate of production of sugars by photosynthesis was almost balanced by their rate of removal by conversion into starch and by translocation.

The total carbohydrate rapidly decreased in the second phase from 6 p.m. until 3 a.m. in consequence of the slowing down and cessation of photosynthesis. Both starch and reducing sugar decreased significantly between 6 p.m. and 9 p.m., but sucrose showed a rapid and significant increase. This increase in sucrose cannot have been directly due to photosynthesis, for more carbohydrate was being removed from the leaf than was formed by photosynthesis. It must be attributed to formation of sucrose from other carbohydrates. Total sugar was almost constant during this period, and it is possible that the sucrose was formed by condensation of reducing sugar. However, this is improbable, since translocation was greater than photosynthesis, and on balance there was a removal of sugar from the leaf. The sucrose must ultimately have come from the hydrolysis of starch, either directly or via reducing sugar. During the period of darkness, from 9 p.m. to 3 a.m., both starch and sucrose fell rapidly, but the change in reducing sugar was small and not significant. The interpretation of this must be either that carbohydrate was translocated in the form of sucrose, or that it was removed in the form of reducing sugar, and the rate of its removal was almost balanced by the rate of production from sucrose and starch.

During the third phase the only significant change was an increase in starch, between 3 a.m. and 9 a.m. It should be noted, however, that immediately after sunrise there was a short period during which starch tended to increase while the sugars, particularly sucrose, tended to fall, and total carbohydrate did not change appreciably. Thus, there was some indication of a condensation of sugars to starch, at this time, when the total carbohydrate content was constant. This was the reverse of the effect observed between 6 p.m. and 9 p.m. These effects occurred at the transition from light to darkness and darkness to light, and there is therefore a suggestion that light influences the dynamical relation between starch and sucrose, in the sense that increasing illumination tends to increase the rate of condensation relative to the rate of hydrolysis. If this interpretation is correct the effect of increased illumination on the starch/sugar relation in the leaf is the reverse of that found in the guard-cells of stomata. Alternatively, it is possible that the changes in the starch/sugar relation were induced by variations in the water content of the leaf. The accumulation of sucrose between 6 p.m. and 9 p.m. corresponded with a water content increasing from low values, and between 3 a.m. and 6 a.m. the reverse change, of a rapid loss of water, was taking place. It has been shown for

detached leaves (10) that starch disappears and sucrose accumulates when the water content falls, but this effect is irreversible, and is in the reverse direction from that found in the present experiment. It is unlikely that temperature could be the operative factor, since it fell only slightly between 6 p.m. and 9 p.m., and there was no appreciable change between 3 a.m. and 6 a.m.

The curve of reducing sugar did not show the irregular fluctuations found by Davis and Sawyer. This may be explained in part by the less frequent sampling, but it suggests that the irregularities were due to sampling errors. Nor was there any inverse correlation between starch and reducing sugars. The two curves varied in the same direction, except for the period between 3 a.m. and 6 a.m., when the changes in neither were significant. Davis and Sawyer also found a positive correlation between the variations in temperature and in sucrose, but this was probably fortuitous. In the present experiment no such correlation was found. Thus, for example, the large increase in sucrose found between 6 p.m. and 9 p.m. occurred at a time when the temperature was rapidly falling.

In so far as these results are relevant to the consideration of the question of the first sugar of photosynthesis, they appear to support the hypothesis that hexose is the first formed sugar. The variations in reducing sugars were regular and significant, and were more closely related than those of sucrose to the changes in starch. Also, sucrose reached its maximum at a later time than starch, and the sucrose accumulated between 6 p.m. and 9 p.m. was undoubtedly a down-grade product.

*(b) The effect of increased KCl supply.*

No significant effect of KCl supply on the starch content of the leaf was found at any time, and this confirms the results of Maskell and James, who found that KCl did not increase the rate of starch production or removal. Nor was there any significant effect of KCl supply on the reducing sugars. On the other hand, addition of KCl caused a highly significant depression of sucrose, but only during the middle of the day, at noon, 3 p.m., and 9 a.m. At other times the depression disappeared. Since none of the carbohydrate fractions were affected during the hours of darkness, it follows that the rate of translocation of carbohydrate during the night was not influenced by variation of KCl supply, as can readily be seen from the curve of total carbohydrate. Thus it is improbable that the depression of sucrose found during the middle of the day was due to increased translocation. It appears more probable that there was an interaction between the effects of increased KCl and some other factor which varied during the day and night. The presence or absence of the KCl effect was not obviously related to the concentration of carbohydrate present in the leaf, nor to temperature. The factor, whose variations

corresponded most closely with the variations in the magnitude of the KCl effect, was the intensity of illumination, and, in fact, the effect was found only during the time of maximum illumination. It is possible, however, that its appearance was determined by low water content, but the considerably higher water content at 9 a.m., when the effect was in evidence, than at 6 p.m., when it was absent, renders this explanation improbable.

The effect of KCl on the rate of photosynthesis presents some peculiarities. If one assumes that the rate of translocation was unaffected, then the depression of total carbohydrate at noon and the absence of any effect at 6 p.m. indicates that the rate of photosynthesis in the intervening period was increased by KCl application. On the other hand, the absence of an effect at 3 a.m. and the significant depression at 9 a.m. indicates that the rate of photosynthesis was decreased by KCl application in this phase.

Any interpretation of the effects of KCl must therefore take into consideration the following circumstances:

(1) The content of starch and reducing sugars was not affected at any time.

(2) Sucrose was depressed during the middle of the day, at the time of maximum illumination and low water content, but not at other times.

(3) The rate of photosynthesis was depressed in the early morning, but was increased in the afternoon.

The most probable explanation appears to be that application of KCl increased the efficiency of the photosynthetic mechanism, and that the apparent reduction in the rate of photosynthesis in the early morning was caused by some secondary effect, possibly later opening of the stomata. The reduction of the concentration of sucrose during the middle of the day, which was not accompanied by a decrease of starch, appears to indicate that at this time an increased supply of KCl caused a shift in the balanced relation of starch and sucrose in the direction of starch.

The water changes give no evidence in favour of the hypothesis of delayed stomatal opening due to application of KCl. Actually the effect of KCl application was to cause a small but not significant increase in the rate of water loss between 3 a.m. and 9 a.m., but this has been shown to be associated with a greater loss of dry matter.

It is difficult to account for the tendency of the residual dry matter to increase over the period of the experiment in plants receiving a low supply of KCl, and to decrease in plants receiving a high supply, without some knowledge of the changes in the constituents of residual dry matter. The explanation possibly lies in a variation in the changes associated with maturation and senescence of the leaf.

SUMMARY.

1. Significant diurnal variations were found in the reducing sugars, sucrose, and starch of potato leaflets. The fluctuations of reducing sugars were small but regular. Sucrose was present in larger amount than reducing sugars and showed greater variation.

2. There was evidence that the change from light to darkness induced a rapid accumulation of sucrose derived from starch, and some indication of the reverse effect in the change from darkness to light.

3. The only significant effect on the carbohydrates estimated of application of KCl was a marked reduction of sucrose during the middle of the day. Starch and reducing sugars were not affected.

4. It appears probable that these effects (2 and 3) were related either to variation of the intensity of illumination, or of water content, but it is not possible to discriminate between these, or other possible causative factors, from the results of the present experiment.

5. The rate of translocation of carbohydrate during the period of darkness was unaffected by application of KCl, but the rate of removal of dry matter was increased.

6. Assuming that the rate of translocation of carbohydrate was also unaffected at other times, the rate of photosynthesis was increased by increased supply of KCl during the afternoon, but was decreased in the early morning. It is suggested that the efficiency of the photosynthetic mechanism was increased by application of KCl, and that the decrease in the early morning was caused by some secondary factor, such as later stomatal opening.

7. The water content per 100 gm. dry matter was increased by application of KCl, and the increase did not vary with time. The loss of dry matter over the sampling period increased with increasing KCl supply, and this was associated with an increased loss of water.

8. The use of the residual dry matter basis of expressing the carbohydrate changes would have given inaccurate results in this experiment, since significant changes in residual dry matter were found.

The author wishes to express his gratitude to Dr. E. J. Maskell and Dr. A. G. Norman for helpful advice on the choice of methods of carbohydrate estimation, to Mr. R. G. Warren for carrying out the  $K_2O$  and Cl determinations, to Mr. F. J. Richards for reading and criticizing the manuscript, and to a number of willing helpers who assisted with the sampling.

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## APPENDIX.

TABLE IV.

*Fresh Weight of Sample of 30 Leaflets.*

Time		Units of KCl				
		0	1	2	3	4
12 noon	B	7.4220	8.7153	9.6193	10.2108	9.7056
	A	7.0606	9.0222	10.7790	12.1333	9.7671
3 p.m.	B	6.8904	9.4269	10.8669	12.7509	9.9644
	A	6.9302	9.2272	9.2990	11.5224	9.7579
6 p.m.	B	6.9102	9.1946	9.6742	11.8827	9.6316
	A	7.3040	8.9471	9.3146	10.6281	9.7967
9 p.m.	B	7.2907	9.0691	9.8186	10.9602	10.0676
	A	7.1801	9.5075	9.2172	11.0807	10.2048
12 midnight	B	7.1470	10.2704	9.5680	10.7819	11.1543
	A	6.9436	8.7724	10.7808	11.6066	10.7324
3 a.m.	B	7.3363	8.8768	10.9221	11.6425	10.6870
	A	7.2765	8.5885	10.0615	11.6759	9.2866
6 a.m.	B	7.2789	8.2017	8.9670	11.1930	9.0293
	A	7.0646	8.6357	9.3369	11.3822	10.4978
9 a.m.	B	7.0989	8.4121	9.1661	10.6698	9.6455
	A	6.6873	9.3716	9.4602	11.8002	8.7000

TABLE V.  
*Dry Weight of Sample of 30 Leaflets.*

Time		Units of KCl				
		0	1	2	3	4
12 noon	B	1.6601	1.8102	2.0191	1.8926	1.8266
	A	1.5870	1.9297	2.2245	2.2306	1.8654
3 p.m.	B	1.4823 (93.40 %)	1.9694 (102.06 %)	2.1190 (95.26 %)	2.3486 (105.29 %)	1.8340 (99.15 %)
	A	1.5582	1.9120	1.8968	2.2634	1.8263
6 p.m.	B	1.5274 (98.02 %)	1.9573 (102.37 %)	1.9173 (101.08 %)	2.2803 (100.75 %)	1.8021 (98.67 %)
	A	1.5857	1.7812	1.8057	2.0413	1.8251
9 p.m.	B	1.4961 (94.35 %)	1.7792 (99.89 %)	1.8344 (101.59 %)	2.0288 (99.39 %)	1.7854 (98.73 %)
	A	1.5231	1.9479	1.7976	2.0340	1.8219
12 midnight	B	1.4043 (92.20 %)	1.9527 (100.25 %)	1.6810 (93.51 %)	1.8104 (89.01 %)	1.7161 (94.19 %)
	A	1.3314	1.5921	1.9239	1.9280	1.8197
3 a.m.	B	1.3919 (104.54 %)	1.5709 (98.67 %)	1.8249 (94.85 %)	1.8420 (95.54 %)	1.6844 (92.56 %)
	A	1.2869	1.4618	1.6290	1.7619	1.4313
6 a.m.	B	1.3931 (108.25 %)	1.4441 (98.79 %)	1.5338 (94.16 %)	1.7726 (100.61 %)	1.4015 (97.92 %)
	A	1.2710	1.4557	1.5529	1.7912	1.5917
9 a.m.	B	1.3958 (109.82 %)	1.5302 (105.12 %)	1.6135 (103.90 %)	1.8280 (102.05 %)	1.5682 (95.52 %)
	A	1.3360	1.7523	1.6339	1.9325	1.4254

Dry weight of each B sample as percentage of the dry weight of the A sample at the previous time is given in brackets.

TABLE VI.  
*Reducing Sugar gm. per 100 gm. Dry Matter.*

Time	Units of KCl.				
	0	1	2	3	4
12 noon	2.51	2.39	2.44	1.43	1.43
3 p.m.	2.25	2.32	2.01	1.94	1.95
6 p.m.	2.26	2.73	2.30	2.37	1.70
9 p.m.	1.78	2.31	1.99	1.88	1.28
12 midnight	1.80	1.91	2.20	2.18	2.07
3 a.m.	1.76	2.18	1.85	1.79	1.83
6 a.m.	1.72	1.85	1.76	1.66	1.53
9 a.m.	2.04	2.16	1.63	1.65	2.17

TABLE VII.

*Sucrose gm. per 100 gm. Dry Matter.*

Time	Units of KCl.				
	0	1	2	3	4
12 noon	4.02	2.47	2.14	1.95	1.85
3 p.m.	3.39	2.89	2.53	1.90	1.75
6 p.m.	2.96	2.93	2.52	1.76	2.97
9 p.m.	3.53	3.35	3.18	2.78	2.99
12 midnight	2.47	2.14	2.42	2.16	2.48
3 a.m.	2.18	2.54	2.46	1.87	2.39
6 a.m.	2.03	1.94	2.05	1.69	2.12
9 a.m.	3.08	2.56	2.12	2.17	1.29

TABLE VIII.

*Starch gm. per 100 gm. Dry Matter.*

Time	Units of KCl.				
	0	1	2	3	4
12 noon	12.01	11.63	10.75	10.58	10.13
3 p.m.	10.98	11.70	8.81	11.17	10.21
6 p.m.	12.71	13.80	12.37	14.42	13.95
9 p.m.	9.29	11.56	10.50	11.21	9.74
12 midnight	6.52	8.94	7.39	7.13	8.36
3 a.m.	4.28	5.03	5.25	4.63	6.01
6 a.m.	5.58	5.83	5.82	5.49	5.84
9 a.m.	6.35	7.13	5.31	6.12	5.77

TABLE IX.

*Water gm. per 100 gm. Dry Matter. (A Samples.)*

Time	Units of KCl.					Mean ( <i>b</i> )	Linear regression coefficient ( <i>c</i> )
	0	1	2	3	4		
12 noon	344.9	367.6	384.6	444.0	423.6	392.9	23.38
3 p.m.	344.8	382.6	390.3	409.1	434.3	392.2	20.55
6 p.m.	360.6	402.3	415.8	420.6	436.8	407.2	17.07
9 p.m.	371.4	388.1	412.8	444.8	460.1	415.4	23.41
12 midnight	421.5	451.0	460.4	502.0	489.8	464.9	18.76
3 a.m.	465.4	487.5	551.6	562.7	548.8	523.2	24.20
6 a.m.	455.8	493.2	501.3	535.5	559.5	509.1	24.97
9 a.m.	400.5	434.8	479.0	510.6	510.4	467.1	29.56
Mean	395.6	425.9	449.5	478.7	482.9	446.5	22.74 ( <i>a</i> )

Standard Errors: (a)  $\pm 2.668$ , (b)  $\pm 5.531$ , (c)  $\pm 3.911$ .

TABLE X.

*Reducing Sugar gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	2.51	2.39	2.44	1.43	1.43	2.040	-0.312
3 p.m.	2.10	2.37	1.91	2.04	1.92	2.068	-0.069
6 p.m.	2.07	2.85	2.21	2.51	1.65	2.258	-0.118
9 p.m.	1.54	2.41	1.95	1.98	1.21	1.818	-0.109
12 midnight	1.43	2.00	2.01	2.05	1.85	1.868	+0.089
3 a.m.	1.47	2.25	1.61	1.60	1.51	1.688	-0.057
6 a.m.	1.55	1.89	1.44	1.50	1.24	1.524	-0.101
9 a.m.	2.02	2.32	1.38	1.52	1.68	1.784	-0.148
Mean	1.836	2.310	1.869	1.829	1.561	1.881	-0.103 (a)

Standard Errors: (a)  $\pm 0.078$ , (b)  $\pm 0.113$ , (c)  $\pm 0.080$ .

TABLE XI.

*Sucrose gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	4.02	2.47	2.14	1.95	1.85	2.486	-0.486
3 p.m.	3.17	2.95	2.41	2.00	1.72	2.450	-0.385
6 p.m.	2.71	3.06	2.43	1.87	2.88	2.590	-0.085
9 p.m.	3.05	3.50	3.11	2.93	2.84	3.086	-0.099
12 midnight	1.97	2.24	2.21	2.03	2.22	2.134	+0.029
3 a.m.	1.82	2.62	2.13	1.68	1.98	2.046	-0.062
6 a.m.	1.83	1.98	1.67	1.52	1.72	1.744	-0.068
9 a.m.	3.05	2.74	1.80	2.00	1.00	2.118	-0.484
Mean	2.703	2.695	2.238	1.998	2.026	2.332	-0.205 (a)

Standard Errors: (a)  $\pm 0.046$ , (b)  $\pm 0.144$ , (c)  $\pm 0.102$ .

TABLE XII.

*Total Sugar gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	6.53	4.86	4.58	3.39	3.29	4.530	-0.795
3 p.m.	5.27	5.32	4.32	4.03	3.64	4.516	-0.455
6 p.m.	4.77	5.91	4.64	4.38	4.53	4.846	-0.201
9 p.m.	4.59	5.91	5.06	4.91	4.05	4.904	-0.208
12 midnight	3.40	4.24	4.22	4.07	4.06	3.998	+0.115
3 a.m.	3.28	4.86	3.74	3.28	3.49	3.730	-0.116
6 a.m.	3.38	3.87	3.11	3.02	2.97	3.270	-0.167
9 a.m.	5.07	5.06	3.17	3.52	2.68	3.900	-0.632
Mean	4.536	5.004	4.105	3.825	3.589	4.212	-0.307 (a)

Standard Errors: (a)  $\pm 0.106$ , (b)  $\pm 0.189$ , (c)  $\pm 0.134$ .

TABLE XIII.

*Starch gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	12.01	11.63	10.75	10.58	10.13	11.020	-0.481
3 p.m.	10.26	11.94	8.39	11.76	10.04	10.478	-0.062
6 p.m.	11.64	14.42	11.91	15.30	13.53	13.360	+0.466
9 p.m.	8.02	12.10	10.27	11.82	9.24	10.290	+0.216
12 midnight	5.19	9.35	6.76	6.69	7.47	7.092	+0.190
3 a.m.	3.56	5.19	4.55	4.15	4.97	4.484	+0.178
6 a.m.	5.03	5.95	4.75	4.95	4.74	5.084	-0.158
9 a.m.	6.29	7.64	4.51	5.63	4.47	5.708	-0.565
Mean	7.750	9.778	7.736	8.860	8.074	8.440	+0.027 (a)

Standard Errors: (a)  $\pm 0.320$ , (b)  $\pm 0.413$ , (c)  $\pm 0.292$ .

TABLE XIV.

*Total Carbohydrate gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	18.54	16.49	15.33	13.97	13.42	15.550	-1.276
3 p.m.	15.53	17.26	12.71	15.79	13.68	14.994	-0.517
6 p.m.	16.41	20.33	16.55	19.68	18.06	18.206	+0.265
9 p.m.	12.61	18.01	15.33	16.73	13.29	15.194	+0.008
12 midnight	8.59	13.59	10.98	10.76	11.53	11.090	+0.305
3 a.m.	6.84	10.05	8.29	7.43	8.46	8.214	+0.062
6 a.m.	8.41	9.82	7.86	7.97	7.71	8.354	-0.325
9 a.m.	11.36	12.70	7.68	9.15	7.15	9.608	-1.197
Mean	12.29	14.78	11.84	12.69	11.66	12.651	-0.334 (a)

Standard Errors: (a)  $\pm 0.416$ , (b)  $\pm 0.534$ , (c)  $\pm 0.378$ .

TABLE XV.

*Dry Matter gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean (b)	Linear regression coefficient (c)
	0	1	2	3	4		
12 noon	100	100	100	100	100	100	—
3 p.m.	93.40	102.06	95.26	105.29	98.32	98.866	+1.307
6 p.m.	91.55	104.48	96.29	106.08	97.01	99.082	+1.252
9 p.m.	86.38	104.37	97.82	105.43	94.90	97.780	+1.810
12 midnight	79.64	104.63	91.47	93.84	89.39	91.794	+0.871
3 a.m.	83.26	103.24	86.76	89.65	82.74	89.130	-1.463
6 a.m.	90.13	101.99	81.69	90.20	81.09	89.020	-2.987
9 a.m.	98.98	107.21	84.88	92.05	77.46	92.116	-5.820
Mean (excluding 12 noon)	89.049	103.997	90.596	97.506	88.701	93.970	-0.719 (a)

Standard Errors: (a)  $\pm 2.389$ , (b)  $\pm 1.238$ , (c)  $\pm 1.036$ .

TABLE XVI.

*Residual Dry Matter gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean ( <i>b</i> )	Linear regression coefficient ( <i>c</i> )
	0	1	2	3	4		
12 noon	81.46	83.51	84.67	86.03	86.58	84.45	+1.276
3 p.m.	77.87	84.80	82.55	89.50	84.64	83.872	+1.824
6 p.m.	75.14	84.15	79.74	86.40	78.95	80.876	+0.987
9 p.m.	73.77	86.36	82.49	88.70	81.61	82.586	+1.802
12 midnight	71.05	91.04	80.49	83.08	77.86	80.704	+0.566
3 a.m.	76.42	93.19	78.47	82.22	74.28	80.916	-1.525
6 a.m.	81.72	92.17	73.83	82.23	73.38	80.666	-2.662
9 a.m.	87.62	94.51	77.20	82.90	70.31	82.508	-4.623
Mean	78.131	88.715	79.930	85.133	78.451	82.072	-0.294 ( <i>a</i> )

Standard Errors: (*a*)  $\pm 1.692$ , (*b*)  $\pm 1.470$ , (*c*)  $\pm 1.039$ .

TABLE XVII.

*Water gm. per 100 gm. Initial Dry Matter.*

Time	Units of KCl.					Mean ( <i>b</i> )	Linear regression coefficient ( <i>c</i> )
	0	1	2	3	4		
12 noon	344.9	367.6	384.6	444.0	423.6	392.94	+23.38
3 p.m.	322.0	390.5	371.8	430.7	427.0	388.40	+25.02
6 p.m.	330.1	420.3	400.4	446.1	423.7	404.12	+21.30
9 p.m.	320.8	405.1	403.8	469.0	436.6	407.06	+29.55
12 midnight	335.7	471.9	421.1	471.1	437.8	427.52	+20.34
3 a.m.	387.5	503.3	478.6	504.5	454.1	465.60	+13.44
6 a.m.	410.1	503.0	409.5	483.0	453.7	451.86	+6.72
9 a.m.	396.4	466.1	406.6	470.0	395.4	426.90	+0.19
Mean	355.94	440.98	409.55	464.80	431.49	420.55	+17.492 ( <i>a</i> )

Standard Errors: (*a*)  $\pm 11.15$ , (*b*)  $\pm 8.73$ , (*c*)  $\pm 6.17$ .



# Cytological Studies of Some Indian Oleiferous Cruciferae.<sup>1</sup> III.

BY

ZAFAR ALAM, M.Sc.

With Plate II.

## I. INTRODUCTION.

CYTOLOGICAL studies were carried out on *Eruca sativa* Lam. (vernacular *taramira*), and four Brassicas. The confusion in the nomenclature of these Brassicas was discussed in Part I. For the reasons given there the following scientific names will be applied to these plants in this paper :

Local Name.	Scientific Name.
<i>Pili</i> (yellow-seeded) <i>sarson</i> .	<i>Brassica trilocularis</i> Hook. fil et Thomps.
<i>Kali</i> (brown-seeded) <i>sarson</i> .	<i>B. campestris</i> L., subsp. <i>campestris</i> var. <i>dichotoma</i> Watt.
<i>Toria</i> .	<i>B. campestris</i> L., subsp. <i>napus</i> var. <i>toria</i> Duth. et Full.
<i>Raya</i> .	<i>B. juncea</i> Hook. fil et Thomps.

## II. MATERIALS AND METHODS.

All the seeds were obtained from the Botanical Experimental Farm, Lyallpur, some of them having been kindly sent by Dr. Abdus Sattar, Ph.D. (London).

For the study of somatic chromosomes the seeds were germinated on damp filter paper. The following fixatives were used :

1. Strong Flemming. 2. Medium Flemming. 3. Navashin. 4. A modification of strong and medium Flemming adopted at the suggestion of Mr. C. S. Semmens, Technical Assistant, Botany Department, King's College, London. In this case osmic acid was replaced by uranic acid of the same strength and proportions. 5. A Flemming modification similar to that recommended by Taylor for smears, and tried with success by Catcheside (5) for some Brassicas.

<sup>1</sup> Part of thesis approved for the Degree of Master of Science in the University of London.



The last fixative was found to be most satisfactory as maltose spaces well the metaphase chromosomes. Sections were cut at  $13\mu$ . The treatment was the same as described for flower-buds.

For the study of meiotic stages the flower-buds were obtained from plants grown in the Experimental Grounds, Regent's Park, London. Before fixation, one of the anthers was examined in Belling's aceto-carmin and only buds showing the requisite division stages were fixed.

The flower-buds were fixed in various fluids.

Pre-treatment with Carnoy's fluid, or chloroform alone, did not give satisfactory results.

Permanent smears fixed in medium Flemming, Benda's fluid, La Cour's 2 BE, and Navashin fluid were also made. Both in the case of flower-buds and smears the best results were obtained with Benda's fluid and medium Flemming. Material was collected between 11 a.m. and 4.30 p.m. on sunny days.

Buds were embedded and cut  $11\mu$  thick. All the preparations were bleached in a three-to-one mixture of 80 per cent. alcohol and hydrogen peroxide (20 vols.) for two to three days, and were stained with a modification of Newton's gentian violet iodine method which gave very satisfactory results. After bleaching, the slides were taken down to 30 per cent. alcohol, wherefrom they were shifted to  $\frac{1}{2}$  per cent. aqueous solution of chromic acid. The following timing was adopted:

0.5 % chromic acid	5 minutes.
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Rinse in water.

Saturated B.D.H. gentian violet	10 minutes.
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1 % iodine and 1 % pot. iodide in 80 % alcohol	1 minute.
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Dehydrate in 95 % and absolute alcohol. Differentiate in clove oil and mount in xylol-balsam.

This modification permitted greater control of the final colour and was used in all cases.

The use of uranic acid instead of osmic acid also gave hopeful results. It was, however, found necessary to bleach the preparations for about 24 hours longer than in the case of osmic acid, and also to treat them with  $\frac{1}{2}$  per cent. aqueous solution of chromic acid for about double the time, viz. 10 minutes. Further tests, however, seem necessary. The uranic acid possesses the advantage of being much cheaper than osmic acid.

The best definition was obtained by examining the preparations under a projector type lamp in a Busch housing with aplanatic condenser and a ground glass screen. A narrow wave-length band was obtained by the use of an Ilford mercury green light filter. A Zeiss aplanatic condenser of N.A. 1.4 was used.

## III. NUMBER, MORPHOLOGY, AND SIZE OF SOMATIC CHROMOSOMES.

The chromosome numbers for all the forms studied are given in Table I.

TABLE I.

*Chromosome Numbers.*

Plant.	Somatic number.	No. of bivalents at meiosis.
1. <i>E. sativa</i> . . . . .	22	11
2. <i>B. trilocularis</i> . . . . .	20	10
3. <i>B. campestris</i> , subsp. <i>campestris</i> var. <i>dichotoma</i>	20	10
4. <i>B. campestris</i> , subsp. <i>napus</i> var. <i>toria</i> . . . . .	20	10
5. <i>B. juncea</i> . . . . .	36	—

The chromosome number has been determined for the first time for *B. trilocularis*. The numbers ascertained for other forms of Brassica confirm the counts for similar forms by Shimotomai (41) and by Manton (28), who has given a list of chromosome numbers which replaces and corrects the list of Tischler (43). The chromosome number ( $2n = 22$ ) for *E. sativa* Lam. is the same as recorded by Manton for *E. sativa* Gars., seeds of which she obtained from South Africa and Stockholm.

The attachment constrictions can be made out in most cases except in a few tiny chromosomes (Pl. II, Figs. 1-5); in the latter they may be terminal. No satellites have been found.

The differences in chromosome length observed are summarized in Table II. The chromosomes in each case were measured in mm. directly from the original drawings and the measurements then reduced to microns. In each case a representative plate was selected for drawing and the measurements given relate to this plate.

TABLE II.

*Chromosome Length* (expressed in microns).

Plant.	Long.	Medium-sized.	Short.
<i>E. sativa</i> . . . . .	2.2	1.3-1.5	1.0-1.2
<i>B. trilocularis</i> . . . . .	2.0	1.3-1.7	1.0-1.1
<i>B. campestris</i> var. <i>dichotoma</i>	2.0-2.3	1.3-1.7	1.0
<i>B. campestris</i> var. <i>toria</i> . . . . .	2.0-2.3	1.3-1.7	1.0
<i>B. juncea</i> . . . . .	1.2-1.3	0.9-1.1	0.7-0.8

It will be seen that of the four Brassicas studied the chromosome length in *B. juncea* ( $2n = 36$ ) is less than in the other three forms ( $2n = 20$ ). A decrease has, therefore, occurred in chromosome size with an increase in the chromosome number. Similar conditions were found by Davie (9) in the genus *Gossypium* and by Gregory (in the press) in the family Zingiberaceae.

## IV. MEIOSIS.

## A. Chromosome Arrangement.

The small size of the chromosomes precludes the possibility of any critical study of the prophase stages. Therefore the observations have been made solely from diakinesis to homotypic telophase.

The observations on *E. sativa* will be dealt with in detail, and any differences observed in the case of the three Brassicas will be pointed out.

(i) *Diakinesis*. At diakinesis there are eleven bivalents in *E. sativa* and ten bivalents in the three Brassicas studied. The bivalents are uniformly distributed (Pl. II, Fig. 6), and there is also repulsion between the members of a bivalent which are held together either at one or both ends. In the drawing, however, it is not possible to show the actual spacing and distribution of all the bivalents, as some of them are situated at different foci. Both ring- and rod-shaped bivalents occur. From mid-diakinesis onwards the chromosomes contract markedly and the two members of a bivalent thicken and shorten, and also come closer to one another. At the same time the bivalents move towards the centre (Pl. II, Fig. 7).

(ii) *Prometaphase*. This converging movement suddenly accelerates, and results in the termination of diakinesis and the very close assemblage of bivalents in the centre of the nuclear area (Pl. II, Fig. 8). The nuclear membrane and the nucleolus disappear. This stage, known as prometaphase, is very short and is characterized by a close grouping of bivalents hitherto widely separated. Kuwada and Sugimoto (24) found differences in staining reactions between chromosomes confined within the nuclear membrane and those free in the cytoplasm. They inferred that chromosomes change from being electro-negative within the nuclear membrane to electro-positive on the spindle. The prometaphase stage, therefore, may be the result of the weakening of the electric charge (which keeps the bivalents well-spaced at mid-diakinesis). Prior to the change to electro-positive sign the repelling force exerted by the poles of the spindle may also contribute towards the close grouping of the bivalents at prometaphase.

(iii) *Metaphase I*. At metaphase I the bivalents assume an arrangement similar to that of floating magnets in an electric field. According to Mayer (29) when the number of floating magnets is 10, three are found in the centre and seven on the periphery in a ring. According to Mizuno (30) and Cannon (4) there are two inside and eight on the periphery. Mayer regards this form as less stable. Kuwada (23), however, has confirmed the arrangement given by Mizuno and Cannon as being stable. All workers are agreed that in the case of 11 magnets, there are three inside and eight on the periphery. An arrangement with two inside and nine on the

periphery has also been reported, but it is unanimously considered to be unstable. In the plants studied both the stable and unstable forms of arrangement have been found at metaphase I (Pl. II, Figs. 9-11). The frequencies of each form of arrangement are summarized in Table III.

TABLE III.

*Frequencies of Forms of Chromosome Arrangement at Metaphase I.*

Plant.	No. of observations.	Frequency of chromosome arrangement.	
		3 inside.	2 inside.
<i>E. sativa</i> ( $n = 11$ ) . . . .	50	49	1
<i>B. trilocularis</i> ( $n = 10$ ) . . . .	50	10	40
<i>B. campestris</i> var. <i>dichotoma</i> ( $n = 10$ ) . . . .	50	8	42
<i>B. campestris</i> var. <i>toria</i> ( $n = 10$ ) . . . .	50	14	36

It is clear that the stable form of arrangement is by far the most frequent. Similar results were obtained by Muto (38) in *Phaseolus chrysanthos* ( $n = 11$ ) and *Cassia occidentalis* ( $n = 13$ ); by Hirayanagi (19) in *Vitis* ( $n = 19$ ); by Ogawa (39) in some Umbelliferae ( $n = 8$  and  $11$ ); and by Maeda and Kato (27) in *Spinacia* and *Vicia* ( $n = 6$ ). In all the forms studied by me the theoretical arrangement is more or less distorted by the effects of secondary pairing, which has taken place between some of the bivalents. A detailed account of secondary pairing is given later. The above workers and Kuwada as well have entirely ignored secondary pairing, which is apparent in most of the plants and is particularly pronounced in *Vitis*. In *E. sativa* it has been found that the form of arrangement resembling that of floating magnets is almost always discernible where four of the bivalents are associated in two groups of twos and the rest are unpaired (Pl. II, Fig. 12); and in some cases even when six bivalents are paired in three groups of twos (Pl. II, Fig. 13). In cases of the secondary association of greater numbers of bivalents, for example, four groups of twos (Pl. II, Fig. 14), the arrangement loses its similarity to that of floating magnets. It is probable, therefore, that some of the configurations found by the above workers, which did not conform to the theoretical arrangement, were due to secondary pairing. As pointed out by Kuwada, some of the anomalies, of course, may also be due to the original orientation of chromosomes distributed in a space of three dimensions, to the viscous nature of the cytoplasm, and to the effects of fixatives, &c., &c.

In order to determine whether the arrangement of bivalents is at random at metaphase I, observations were made on 25 plates with regard to the relative position of the three unpaired bivalents in plates possessing the 3(1) + 4(2) type of secondary pairing. These three unpaired bivalents,

which are free from the effects of superimposed secondary pairing, can be arranged in the following three ways:

(a) All the three bivalents near to one another.

(b) Two of the three bivalents near to one another, the other widely separated.

(c) All the three bivalents separate from one another.

If the arrangement is at random the above three types should occur in a 1:3:1 ratio. The frequencies of arrangement observed and expected in the 25 cases are given in Table IV.

TABLE IV.

*Frequencies of Arrangement of Three Unpaired Bivalents at Metaphase I.*

	All three near to one another.	Two near to one another.	All three separate from one another.
Observed	5	15	5
Expected	5	15	5

The observations agree perfectly with the expectation, showing that the arrangement is at random, at least in the case of chromosomes free from the effects of secondary pairing. Presumably the same condition exists at all the stages studied.

(iv) *Anaphase I.* Kuwada assumes that the two poles of the spindle attract the chromosomes, and the anaphasic separation is solely due to this attraction. Darlington (7), on the other hand, assumes that the poles repel the chromosomes and considers this repulsion essential for the equilibrium at metaphase. Following Bělař (1-3), he attributes the anaphasic separation mainly to the narrowing and axial stretching of the spindle without change in bulk.

By floating eleven pieces of cork with magnetized needles stuck in them, in a beaker of water, I found that the usual magnetic form of arrangement (three inside and eight on the periphery) was obtained only when the magnet placed over the top bears a charge opposite to that of the needles. The distance had, of course, to be regulated, but the fact remains that equilibrium was established. A magnet of the same charge as the needles dispersed the corks, which arranged themselves in a ring at the sides of the beaker. It therefore appears that Kuwada's supposition of the poles carrying charges opposite to the chromosomes is more reasonable. Kuwada's explanation of the anaphasic separation being entirely due to the attraction force from the poles is, however, open to certain objections, as pointed out by Darlington. Darlington's explanation also seems open to criticism, as the repulsion from the poles would increase with the proximity of the

chromosomes to the poles, and therefore quite a strong force would be required to push the chromosomes to the poles in the face of this opposition. Furthermore, his hypothesis would not account for the clumping of chromosomes at the end of anaphase in the so-called 'tassement polaire'. It was probably these considerations which led Darlington to assume that the repulsion force from the poles weakens during anaphase.

I am inclined to think that the anaphasic separation is the result of more than one force. The poles attract the chromosomes, but this attraction is just enough to keep them in equilibrium and is not strong enough to pull them apart. At early anaphase the attachment constrictions repel one another and thus push the chromosomes apart (as suggested by Darlington). At mid-anaphase the stretching of the spindle separates them still further. Lastly, the force of attraction from the poles of the spindle (which would be stronger, due to the chromosomes being nearer to the poles) pulls the chromosomes to the poles. On this hypothesis, anaphasic separation is therefore the result of (1) the repulsion between attachment constrictions, (2) the axial narrowing and stretching of the spindle, and (3) the attraction from the poles. If any of these forces fails to function, the anaphasic separation does not take place. The explanation offered above, like all others, is only tentative, but it possesses the advantage of explaining most of the phenomena which it would not be possible to explain according to Kuwada's or Darlington's views alone.

Lagging bivalents are occasionally found at anaphase I in *E. sativa* (Pl. II, Fig. 15) and the three Brassicas studied. Kuwada attributes lagging to an insufficiency of the electric charge carried by the lagging chromosomes, which, therefore, cannot move with the same speed through the spindle as others carrying a higher charge. This is, however, based on the unproved premiss that the charge carried by certain chromosomes varies from cell to cell, as lagging is not met with in every cell. Catcheside, on the other hand, has attributed lagging to the presence of interstitial chiasmata. His explanation fits in better with the conditions found in these plants.

(v) *Interkinesis*. After the chromosomes have separated to opposite poles, each group organizes a telophase nucleus. The nucleolus appears; the interkinesis is fairly well marked, and the chromosomes are uniformly distributed as at diakinesis (Pl. II, Fig. 16).

(vi) *Homotypic divisions*. Interkinesis is followed by prometaphase II. At metaphase II (as at metaphase I) the chromosomes assume the arrangement of floating magnets (Pl. II, Fig. 17). The secondary pairing again distorts this arrangement (Pl. II, Fig. 18). At anaphase II the conditions are similar to those at anaphase I. At telophase II the distribution of chromosomes is similar to that found at interkinesis and diakinesis.

Gates (12) first observed the uniform spacing of bivalents, in *Oenothera*,

at diakinesis and interkinesis, and concluded that the chromosome bivalents are mutually repelled. He concludes: 'All these facts point to the supposition that the chromosome bivalents are mutually repelled. It is true that in early telophase the chromosomes form a close group, so that they certainly cannot be repelled at that time, but may be attracted. However, the medium in which bodies float frequently changes their qualities of attraction and repulsion, and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float.'

### *B. Secondary Pairing.*

In all the plants studied a variable number of bivalents are found to be secondarily paired. Secondary pairing may be described as a generalized attraction between chromosomes phylogenetically, though distantly, related. It appears to be due to a residual attraction between chromosomes in which ancestral homology is still discernible, and is an indication of polyploidy. Lawrence (26), who has given a general review of the problem, considers that the secondary association is a phenomenon intimately connected with allopolyploidy, and that the degree of association can be used as a measure of the phylogenetic age of the form under examination. It can in many cases be used as a measure of quantitative and perhaps structural changes in the complement of chromosomes. It is different from prophasic pairing, which shows a closer relation between the chromosomes. According to Sansome and Philp (40), it is analogous to the generalized attraction between homologous pairs of chromosomes at mitosis (somatic pairing), which occurs in some organisms. In the somatic tissue of the plants studied (Pl. II, Figs. 1-5) a tendency for the chromosomes to be associated either in pairs or in greater numbers to form a group has been observed. Similar conditions were observed by Skovsted (42) and Davie (9) in cotton and certain other Malvaceous plants. As stated by Davie, 'it is reasonable to suppose that "somatic pairing" would be observable in organisms showing secondary pairing at meiosis, since the chromosomes do not lose their segmental homology when they appear in the diploid chromosome complement'.

In meiosis the conditions are particularly favourable for secondary association, as the chromosomes are brought within the range of attraction (which appears to be limited) by assemblage in a group at prometaphase. The size also must have an effect, as the smaller chromosomes would be more easily moved than the larger ones. It is because of this fact that secondary pairing is not found in polyploids with large chromosomes. After prometaphase there ensues a repulsion, the secondarily paired chromosomes remaining in close association till telophase. Secondary pairing has been found to exist both in heterotypic and homotypic metaphases and anaphases.

In the plants studied it was found that there were no connexions between the secondarily paired chromosomes, nor did they touch except through collapse in fixation. This is in accordance with the general view. Gustafsson (18), however, has endeavoured to show that if the attraction is strong enough the chromosomes may touch or even fuse. He states: 'Now inasmuch as the homology between different pairs of chromosomes or univalents varies from case to case (as in *Taraxacum*) there will therefore arise a variation in the attraction. On the one extreme we have those cases in which the chromosomes lie freely in the vicinity of one another without touching, and on the other extreme those cases in which they touch one another or are even fused.' He has not given any description of the fixatives used or the technique. I understand, however, that *Taraxacum* is difficult to fix, and it is probable that poor fixation may have produced the extreme effects of contact or fusion found by him to occur.

#### 1. *E. sativa* Lam.

A considerable amount of secondary pairing occurs in *E. sativa*. The number of secondary associations per plate in metaphase I ranges between 1 and 5, the mode being at 3-4 (Table V). If the secondary pairs of bivalents are each considered as one body, then the number of bodies in a plate has never been found to be less than 6. Therefore the basic chromosome number is  $b = 6$ . The letter  $b$  is used in this paper as a symbol for the basic number as suggested by Gates (14).

TABLE V.

*Number of Secondary Associations in Polar View of Metaphase I Plates.*

No. of secondary associations per plate	1	2	3	4	5	Total.
No. of plates . . . . .	2	9	30	30	19	90

(In determining the number of secondary associations two paired bivalents are considered to have one, and a group of three bivalents two secondary associations.)

The usual pairing consists of groups of two bivalents (Table VI), the maximum being 1(1) + 5(2) (Pl. II, Fig. 19). Sometimes, however, three bivalents have been found to be secondarily paired (Pl. II, Fig. 20), but in no case has more than one group of three secondarily paired bivalents been found in the same cell (Table VI). In all cases where the maximum pairing consisting of 1(1) + 5(2) was found, two groups each consisting of two secondarily paired bivalents were always close to one another (Pl. II, Fig. 19). This suggests an affinity (due most probably to structural change resulting in different distribution of homology) between at least one of the bivalents in each group. This affinity, however, does not seem to be strong enough to pull the two groups together to form a group of four bivalents. This



weak affinity may, therefore, result in the secondary pairing of the weakly homologous chromosomes in the absence of competition. Now if one of these had already paired with its mate to give a group of two, and the other one happened to be away from the bivalent for which it has greater affinity, but within the range of attraction of its weakly homologous bivalent, it might pair with the latter to give a group of three bivalents.

The haploid chromosome set of *E. sativa* (assuming that structural changes have occurred) may, therefore, be represented as follows:

AA BB CC DD EE F

No genetical work seems to have been done so far on *E. sativa*, but the above constitution provides a basis for multiple (duplicate) factors, and therefore 15:1 ratios may be expected.

TABLE VI.

*Types of Secondary Association at Meiosis.*

No. of secondary associations.	Nos. of bivalents in association.			No. of cases.	Total.
	1	2	3		
1	9	1	0	2	2
2	7	2	0	9	9
3	5	3	0	30	30
4	3	4	0	25	30
	4	2	1	5	
5	1	5	0	12	19
	2	3	1	7	
Grand total					90

Table VI summarizes the different types of secondary association, together with the frequency of each. It is clear that the modal frequency is 3 and 4, i.e.  $\frac{3}{5}$  and  $\frac{4}{5}$  of the possible secondary associations are most frequently developed.

It has not been found possible to analyse fully the whole complement in any one cell in profile view at metaphase I owing to the secondarily paired groups lying obliquely or perpendicularly. The examination of early anaphase I, however, is very instructive from this point of view. Pl. II, Fig. 21 a and b show two anaphase groups in two foci from the same cell, and Fig. 22 gives a diagrammatic representation of the metaphase as it would have appeared in profile view.

As already stated, secondary association occurs in both heterotypic and homotypic metaphases and anaphases, and it does not affect disjunction (Pl. II, Figs. 18, 19, 20, 21 a and b, 23 a and b). As previously mentioned, lagging bivalents are occasionally found at anaphase I.

The derivation of the chromosome complement of *E. sativa* from  $b = 6$  may be explained as follows. Suppose in the original type the constitution of the haploid set of six chromosomes was represented by A. By gene mutation or structural changes such as translocation, inversion, interchange or reduplication, another type having a different constitution, say A', may arise from the original. A cross between A and A' would be at least partially sterile due to the low frequency of pairing. A similar case has been observed by Goodspeed and Clausen (16) in the cross *Nicotiana Bigelovii*  $\times$  *N. suaveolens*, where 0-1 bivalents were formed out of twenty-four chromosomes.

Now by the occurrence of amphidiploidy in the cross  $A \times A'$  (cf. *Raphanus sativus*  $\times$  *Brassica oleracea*, Karpechenko (20) the tetraploid number twenty-four would be obtained. Fusion of four chromosomes to give two would result in the production of the 22-type found in *E. sativa*. If the supposition of the fusion is correct then two chromosomes should be longer than the rest in the somatic tissue, and one bivalent larger than the others in metaphase I. In somatic tissue I actually find two chromosomes longer than the rest (Pl. II, Fig. 1), These two chromosomes (cf. Table II) are  $2.2\mu$  long, while the rest range from 1.0 to  $1.5\mu$ . In metaphase I, however, I have not been able to locate the difference conclusively.

The above explanation appears to be plausible, as doubling of the chromosome number gametically has been observed in an inter-generic hybrid in Cruciferae (*Raphanus-Brassica*, Karpechenko (20, 21)). According to this explanation an allopolyploid would be produced directly. On the other hand, however, there is also the possibility of a non-hybrid doubling its chromosome number and producing an autotetraploid. Subsequent structural changes and gene mutations in an autopolyploid would give allopolyplody eventually.

Alternatively, a secondary balance of eleven from a basic number of six might have been reached through unbalanced multiplication by which five of the basic chromosomes were doubled and one not doubled.

2. *Brassica trilocularis*, *B. campestris* var. *dichotoma*, and *B. campestris* var. *toria*.

As already stated, ten bivalents are found at diakinesis. At metaphase I the bivalents show quite marked secondary pairing. They also show size variations. The maximum secondary pairing observed in each of these plants is shown in Pl. II, Figs. 24-6. The extent of this as seen in polar view, and the various types of secondary association, are summarized below in Tables VII and VIII.

TABLE VII.

*Extent of Secondary Associations.*

Plant.	No. of polar metaphase I plates possessing the following numbers of secondary associations.				Total.
	1	2	3	4	
<i>B. trilocularis</i> . . . . .	4	13	29	8	54
<i>B. campestris</i> var. <i>dichotoma</i>	13	19	21	17	70
<i>B. campestris</i> var. <i>toria</i> . .	6	15	23	7	51
Grand total					175

The number of secondary associations ranges between one and four, the mode being at three in each case.

TABLE VIII.

*Types of Secondary Association in Metaphase I.*

No. of secondary associations.	No. of bivalents in association.			No. of cases.	Total.
	1	2	3		
1	8	1	0	23	23
2	6	2	0	46	47
	7	0	1	1	
3	4	3	0	67	73
	5	1	1	6	
4	2	4	0	22	32
	3	2	1	10	
Grand total					175

It is clear from the results that the modal frequency is three. The number of associations has never been found to exceed four. The types of association found at this maximum in each of the three plants studied is given in Table IX.

TABLE IX.

	No. of cases with maximum association.	
	2 (1) + 4 (2)	3 (1) + 2 (2) + 1 (3)
<i>B. trilocularis</i> . . . . .	8	nil
<i>B. campestris</i> var. <i>dichotoma</i>	14	3
<i>B. campestris</i> var. <i>toria</i> . .	nil	7

In none of the metaphase plates studied was more than one group of three bivalents observed.

A possible composition of *B. trilocularis* from  $b = 6$  could be arrived at by supposing that it is tetrasomic with regard to four of the basic set, with the possibility of slight structural changes having taken place. *B. campestris* var. *dichotoma* appears to be of the same composition, but the structural changes in this case have taken place to a greater degree than in *B. trilocularis*. In *B. campestris* var. *toria* only one type of maximum association, viz.  $3(1) + 2(2) + 1(3)$ , has been found to occur in metaphase I, the other

type, viz. 2(1) + 4(2), being absent in the plates studied. The latter type of association, however, has been observed in metaphase II (Pl. II, Fig. 27). It would be interesting to compare the types of association at metaphase I and II. *B. campestris* var. *toria* has probably the same constitution as the other two plants, but structural changes must have occurred to a much greater extent. The differences in the time of growth, &c., may perhaps be responsible to a certain degree in hastening the structural changes, or it may be that *B. campestris* var. *toria* is more ancient than the other two plants. Another point that has been noted in *B. campestris* var. *toria* is the association of chromosomes of different sizes, indicating possible structural changes in its ancestry.

#### V. DISCUSSION.

Secondary pairing was first observed by Kuwada (22) in *Oryza sativa* L. Subsequently it was noted by a number of workers in other plants, but its significance in regard to the homology of associated bivalents was not recognized till recently (6). Later, Darlington and Moffett (8) utilized it for tracing the historical relationships in Pomoideae, and showed that the chromosome number 17 was derived from  $b = 7$ . As stated by Gates (15), secondary pairing has been observed in *Pyrus*, *Dahlia*, *Brassica*, *Gossypium*, and many other plants.

Secondary pairing, as recognized in this paper, is based on the conception that different pairs of chromosomes bear factors, or more likely, blocks of factors in common. The existence of duplicate factors is well known in *Brassica*; the cytological basis has been shown to be polyploidy, which is frequently secondarily balanced. This condition may permit duplicate factors in some parts of the chromatin, but not in others.

The cytological studies carried out by Morinaga (31-6) on some interspecific hybrids of *Brassica* can be employed to some extent for tracing the relationships and for drawing some preliminary conclusions. His results are summarized briefly in Table X.

TABLE X.

*Summary of Morinaga's Results on Interspecific Hybridization in Brassica.*

Haploid number of parents.	No. of bivalents.	No. of univalents.
10 × 10	10	—
10 × 17	1-9	25-9
10 × 18	10	8
10 × 19	10	9
18 × 19	10	17
18 × 8	8	10

Morinaga (36) has recently discussed his results. The species having  $n = 10, 8$ , and  $9$  he calls 'elemental species' I, II, and III respectively,

and considers the species with higher chromosome numbers to be amphidiploid from the cross between two of these elemental species. For example, according to him, species with  $n = 18$  are amphidiploid from elemental species I and II. But, as suggested by Catcheside (5),  $n = 18$  can also be derived by amphidiploidy from a cross between species having nine as haploid number.

From the summary given in Table X it is evident that, assuming allosyndesis to have taken place, almost all the species have one or other particular group of chromosomes in common. This evidence gives an idea of the differences in the chromosome complement of the various species, without specifying, however, whether these differences are qualitative or quantitative. In a cross between species with haploid numbers 18 and 10, in which ten bivalents and eight univalents occur, Morinaga assumes that ten chromosomes of the latter species have paired with ten chromosomes of the higher-numbered species. This assumption is not fully justified, as it is based on the unproved assumption that autosyndesis does not take place.

Catcheside studied the cytology of the  $F_1$  of a cross between swede ( $n = 18$ ) and turnip ( $n = 10$ ). He found 7-12 bivalents and 1-3 trivalents at meiosis. He also found that secondary pairing was more pronounced in the hybrid than in the turnip. In fact, the condition ten bivalents and eight univalents, reported by Morinaga as of universal occurrence in a  $18 \times 10$  cross, was found to occur only twice in the seventeen cells examined by Catcheside. It therefore appears that the situation is much more complex than Morinaga has suggested.

In cases where secondary pairing exists it is possible that some bivalents may be formed in a haploid plant. Morinaga and Fukushima (37) found 0-6 or 7 bivalents at metaphase I in a haploid mutant of *Brassica napella*. This intra-haploid homology furnishes evidence of polyploidy. If the 'non-homologous' chromosomes of the haploid set consist of series of particles (genes) *abcdef...* and *mnopq...* then in the presence of identical mates the *a* particles of different pairs will not pair, but in a haploid, due to the absence of competition, they may often pair and result in the formation of bivalents. In a diploid, however, such short reduplicated segments may result in secondary pairing. I actually find some clear cases of secondary pairing in photomicrographs of metaphases I and II in the diploid *B. napella* (Morinaga, 1929 a, Pl. II, Figs. 41 and 42). Since it is probable that Morinaga has selected only those plates in which the chromosomes were best spread, and therefore least secondarily paired, the average amount of pairing would be higher than this. As already stated, reduplication in a diploid would not interfere with normal pairing. Rarely, however, by an unusual association of the chromosome pairs of different homologies carrying short blocks of identical genes a certain amount of autopolyploid pairing may occur.

Catcheside has reviewed the work of Manton (28), and I have nothing to add to it, except that a phylogenetic fusion of chromosomes has been indicated in *E. sativa*. I agree with Catcheside that the changes called by Manton aneuploid changes (loss or gain) are more probably due to fusion, fragmentation, and change in balance.

Various basic chromosome numbers, viz. 8, 9, 10, 17, 18, and 19 (Morinaga and others) have been reported for different Brassica species. It is probable that all these are secondarily balanced numbers, and perhaps represent different balances of the same primary number. The occurrence of this wide and more or less continuous range of haploid numbers in Brassica indicates crossing with the subsequent establishment of a variety of derived secondarily polyploid types. In all the plants studied  $b = 6$ , but the types of association suggest superimposed structural changes.

In *E. sativa* it is suggested that the variation in number is due to fusion or unbalance, and it is probable that similar phenomena may have occurred in the Brassicas as well. In addition, fragmentation of chromosomes also may have occurred and increased the chromosome number. This can be ascertained from studies of somatic chromosomes, but their small size would considerably hamper progress in this direction.

A systematic study of the various Cruciferous plants from a genetical and cytological standpoint (as suggested by Gates (13) for Malvaceae) should supply clues to the origin and phylogeny of the Cruciferae.

## VI. SUMMARY.

1. The *somatic* chromosome numbers have been ascertained for the following plants, and corroborated by meiotic counts except in the case of *B. juncea*.

<i>E. sativa</i> Lam.	22
<i>B. trilocularis</i> H.f. and T.	20
<i>B. campestris</i> var. <i>dichotoma</i> Watt.	20
<i>B. campestris</i> var. <i>toria</i> D. and F.	20
<i>B. juncea</i> H.f. and T.	36

A comparison of the relationships of the length and number of somatic chromosomes of the four Brassicas has shown that the length decreases with increase in number and *vice versa*.

2. The microsporogenesis has been studied with special reference to chromosome arrangement and secondary pairing.

3. The bivalents are uniformly distributed in the nucleus at diakinesis. The arrangement at interkinesis and telophase II is similar to that at diakinesis.

At metaphase the chromosomes assume an arrangement similar to that of floating magnets in an electric field. The stable form of arrangement

has been found to be most frequent. Secondary pairing beyond a certain limit distorts this arrangement.

4. It is suggested that the anaphasic separation is the result of (i) repulsion between attachment constrictions, (ii) axial narrowing and stretching of the spindle, and (iii) attraction from the poles.

5. Secondary pairing occurs in both heterotypic and homotypic metaphases and anaphases. The minimum number of groups of bivalents has been found to be six, which is, therefore, the primary basic chromosome number.

In *E. sativa* the variation in number is probably due to fusion of two pairs of chromosomes or to unbalance. *B. trilocularis*, *B. campestris* var. *dichotoma*, and *B. campestris* var. *toria* are probably secondarily balanced polyploids with superimposed structural changes. Additional evidence of polyploidy in Brassica is supplied by the well-known occurrence of duplicate factors.

6. The phylogeny of the Cruciferae appears to have involved cases of fusion, fragmentation, reduplication, and other structural changes besides polyploidy and change in balance.

In conclusion, I must express my sincerest thanks to Professor R. Ruggles Gates, F.R.S., for sympathetic guidance, valuable criticism and adequate facilities in connexion with these investigations. I am also indebted to Mr. D. G. Catcheside for helpful suggestions.

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## EXPLANATION OF PLATE II.

Illustrating Mr. Zafar Alam's paper on 'Cytological Studies of Some Indian Oleiferous Cruciferae'.

Drawings were made with the aid of  $\frac{1}{12}$  in. oil immersion Busch objective and a Reichert camera-lucida with ocular  $\times 20$ , giving a magnification of 3,000.

Figs. 1-5. Somatic chromosomes in polar metaphase. Fig. 1. *Eruca sativa* Lam. ( $2n = 22$ ). Fig. 2. *Brassica trilocularis* Hook. fil et Thomps. ( $2n = 20$ ). Fig. 3. *B. campestris* L., subsp. *campestris* var. *dichotoma* Watt ( $2n = 20$ ). Fig. 4. *B. campestris* L., subsp. *napus* var. *toria* Duth. et Full. ( $2n = 20$ ). Fig. 5. *B. juncea* Hook. fil et Thomps. ( $2n = 36$ ).

Figs. 6 & 7. Pollen mother-cell nuclei at diakinesis in *Eruca sativa*. Fig. 6. Mid diakinesis. Fig. 7. Late diakinesis.

Fig. 8. Prometaphase in *E. sativa*.

Figs. 9-11. Plates at polar metaphase I showing forms of magnetic arrangement. Fig. 9. Stable form of arrangement in *E. sativa*. Fig. 10. Stable form of arrangement in *B. trilocularis*. Fig. 11. Unstable form of arrangement in *B. trilocularis*.

Figs. 12-14. Plates of polar metaphase I showing varying degrees of secondary pairing in *E. sativa*. Fig. 12.  $7(1) + 2(2)$ . Fig. 13.  $5(1) + 3(2)$ . Fig. 14.  $3(1) + 4(2)$ .

Fig. 15. Pollen mother-cell showing lagging bivalent at anaphase I in *E. sativa*.

Fig. 16. Interkinesis in *E. sativa*.

Figs. 17 and 18. Pollen mother-cells at polar metaphase II in *E. sativa*. Fig. 17. Stable form of magnetic arrangement. Fig. 18. Magnetic arrangement distorted by secondary pairing, the pairing being  $3(1) + 4(2)$ .

Figs. 19 and 20. Plates of polar metaphase I showing secondary pairing in *E. sativa*. Fig. 19. Maximum association,  $1(1) + 5(2)$ . Fig. 20. A group of three secondarily paired bivalents,  $4(1) + 2(2) + 1(3)$ .

Fig. 21. Early anaphase I in *E. sativa* showing persistent secondary pairing. a. upper focus, b. lower focus.

Fig. 22. Diagrammatic representation of the metaphase I condition of figs. 21 a and b as seen in profile.

Fig. 23. Early anaphase II in *E. sativa* showing persistence of secondary pairing. a. upper focus, b. lower focus.

Figs. 24-6. Polar metaphase I plates showing maximum secondary association. Fig. 24. *B. trilocularis*,  $2(1) + 4(2)$ . Fig. 25. *B. campestris* var. *dichotoma*,  $2(1) + 4(2)$ . Fig. 26. *B. campestris* var. *toria*,  $3(1) + 2(2) + 1(3)$ .

Fig. 27. Metaphase II, showing maximum association in *B. campestris* var. *toria*,  $2(1) + 4(2)$ .





# An Interpretation of the Morphology of the Single Cotyledon of *Ranunculus Ficaria* based on Embryology and Seedling Anatomy.

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With thirty-eight Figures in the Text.

## INTRODUCTION.

THE seedling of *Ranunculus Ficaria* is remarkable among the Ranunculaceae since normally it possesses only one cotyledon (Fig. 1). This is a petiolate organ similar to the foliage leaves, except that the lamina is generally bilobed. The depth of the apical indentation in the lamina varies considerably. The upper portion of the petiole immediately below the point of attachment to the lamina is grooved on one side. The greater part of the petiole, however, is circular in cross-section, but at the base it forms a sheath which at an early stage surrounds the stem apex and embryonic leaves. It thus follows that the first foliage leaf and all the succeeding foliar members arise at higher levels on the axis than does the single cotyledon.

Since the middle of the last century the morphology of this single cotyledon has been a subject for speculation amongst botanists. On the one hand there are those like Sterckx (9) in 1900 and Sargent (6) in 1903, who considered that it is a syncotylous organ, equivalent to two original cotyledons fused together along one margin of the lamina and throughout the length of the petioles. Irmisch (5), one of the first botanists to examine the cotyledon of *R. Ficaria*, at first believed it to be syncotylous, but as early as 1854 he had already changed his mind, and thought it was to be regarded as a single organ comparable with a single member of a pair of cotyledons. Schmid (7) in 1902, in an account of the embryology of various pseudo-monocotyledons, was content to record that in *R. Ficaria* he could find no trace of the second cotyledon. H. Winkler (11, 12), in 1926 and 1927, first refers to the view expressed by A. Winkler in 1885, that there is no true cotyledon in *R. Ficaria*, and that the apparent cotyledon represents the first foliage leaf although differing in shape from the others. Winkler, however, dismisses this suggestion with the observation that it is

not generally accepted by modern botanists, and gives his reasons for believing that the cotyledon is a single organ, and that the second one fails to develop. Winkler's papers, however, incited Bugnon (1) in 1933 to state his belief that *R. Ficaria* is heterocotylous, the first foliage leaf being homologous with the second cotyledon. A similar view has also been expressed by Zimmermann (14) in 1935, who, in the course of a long paper on phyllotaxy, says of *R. Ficaria*: 'Das nächste Blatt (first foliage leaf) steht zwar noch wie ein typisches Keimblatt in einer näher an 180° herankommenden Divergenz zum Kotyledon, hat aber in der Regel die Fähigkeit Kotyledon zu werden, verloren.' Weisse (10), in 1930, however, gave a description of the seedling anatomy which makes this suggestion highly improbable, but it is remarkable that Zimmermann refers to this paper as being in favour of his views.

This brief recapitulation shows the great variety of speculations concerning the morphology of the cotyledon of *R. Ficaria*. This, in the main, is because the different views that have been put forward have been based on incomplete investigations. Before proceeding to describe the present work it will be necessary to consider more closely these previous studies in order to assess the value of the theoretical views to which they have given rise.

Information concerning the morphology of the cotyledon can be obtained by studying the macroscopic features, the anatomy and the development of the seedling, and the development of the embryo. All these methods have previously been applied to *R. Ficaria* by different investigators, but there does not appear to be a single instance in which all these methods have been employed by the same investigator. Irmisch, who gives small but good figures of the seedlings in his paper, discarded the view that the single cotyledon is syncotylous; (1) because he frequently found trilobed cotyledons; (2) because there is frequently a large vein from the top of the petiole to the base of the apical indentation of the lamina, and (3) because the vascular anatomy of the petiole cannot readily be explained on a syncotylous basis. Sterckx, as a result of embryological and comparative morphological work on various Ranunculaceae, was so firmly convinced that the single cotyledon of *R. Ficaria* is syncotylous that he does not appear to have believed any other morphological interpretation to be possible. He arrived at this conclusion chiefly as a result of observing amongst the Ranunculaceae different species showing various grades of concrescence along both margins of the cotyledonary petioles so as to form a 'tube'. Hence it was easy to imagine that in *R. Ficaria* the two original cotyledons had fused along one margin. As additional evidence in favour of this view, he pointed out that the upper part of the cotyledonary petiole is grooved, and that the lamina shows various stages of bifurcation in different individuals.

This paper by Sterckx appears to have exercised a considerable influence on Sargent, who, as a result of prolonged and careful researches on the seedling anatomy of a wide range of species, put forward the theory that the single cotyledon of all true Monocotyledons was derived phylogenetically by the fusion of the two cotyledons characteristic of most Dicotyledons. It is important to note that Miss Sargent's arguments are based exclusively on the vascular anatomy of the seedlings, and that no other mode of approach to the subject was employed. In the course of her work she examined the seedlings of various species of Ranunculaceae, including some with cotyledons partly concrescent along both margins of the base of the petiole so as to form a 'tube', and afterwards deduced, like Sterckx, that in *R. Ficaria* the two original cotyledons had become fused along one margin only, so that the cotyledon is to be regarded as syncotylous. She states that in *R. Ficaria* 'a single massive bundle runs down the whole length of the cotyledonary petiole . . . at the base of the cotyledon the petiole is bordered by two membranous wings which are united round the embryonic bud of the plumule into a closed sheath. *At this level the cotyledonary trace has opened out slightly: the phloem mass is divided as well as the metaxylem.*' The 'opening out' of the single vascular strand is taken to be an ancestral character which, by persisting, provides evidence that the single vascular strand has developed phylogenetically from two which have fused together. During the present investigation it has been found that although many cotyledonary petioles of *R. Ficaria* are provided with only one vascular strand as described by Miss Sargent, many others have one large median strand and two smaller lateral ones. The figures in Weisse's paper (10) likewise show the cotyledonary petioles with three bundles. In the course of my work it has been possible to re-examine Miss Sargent's original slides, which were kindly sent on loan from Girton College, Cambridge. These slides consist of serial sections of three seedlings of different ages, and in all of them there is only one vascular strand in the cotyledonary petiole. In one of the seedlings the cotyledonary bundle is stained in such a way that the xylem appears to be divided into two masses, but even so there is a single sheath surrounding the bundle, and the grounds for believing it to represent two fused bundles are by no means convincing. This, as was to be expected, shows that Miss Sargent's observations were accurate so far as the material on which she worked is concerned. Dr. A. Arber has pointed out to me that at the date when Miss Sargent's work was done there was little knowledge of variation in seedlings, and it seemed reasonable to suppose that, if one examined three series of sections through seedlings of more than one age, and the results agreed in detail, there would be no need to go further.

The most informative papers on the subject which have appeared in recent years are those by H. Winkler, in which he records the existence of

and figures seedlings with two cotyledons, each of which is bilobed. Because there is usually a main vein extending to the base of the apical indentation of the lamina (this is denied by Miss Sargent, but appears to be generally true of the considerable number of seedlings which have been examined during the present investigation), and also because trilobed cotyledons are sometimes found, Winkler does not believe there is any evidence of syncotyly. He states definitely that in his opinion the second cotyledon has become lost. Bugnon's objections to this opinion of Winkler's are based largely on macroscopic observations on seedlings. Bugnon's and Zimmermann's suggestion that the first foliage leaf is homologous with the second cotyledon cannot be accepted, however, because Weisse found that in some dicotyledonous seedlings of *R. Ficaria* supplied by Winkler, the two cotyledons arose opposite to one another at the same level on the axis, and had the bases of their petioles partly concrescent so as to form a short tube. He says: 'Die abnormerweise zur Entwicklung gekommenen zwei Kotyledonen standen auf gleicher Höhe genau gegenüber und waren mit ihren Rändern zu einer kurzen Scheide verwachsen.' Hence it is unreasonable to argue that the first foliage leaf represents the missing cotyledon in monocotylous seedlings, because the first foliage leaf arises at a higher level on the axis, and is at first surrounded by the base of the single organ. In addition, the present work on the embryology of *R. Ficaria* has revealed what is believed to be the rudimentary second cotyledon which fails to develop. This likewise does not favour the suggestion that the first foliage leaf is homologous with the second cotyledon.

#### METHODS.

There are two main difficulties in studying the embryology and seedling anatomy of *R. Ficaria*: (1) that viable seed is not readily obtained; (2) that if viable seed is sown towards the end of the summer of the year in which it is formed, either in a greenhouse or out of doors, the young seedlings do not appear above ground until about February or March of the following spring. The causes of the frequent sterility, which has already been the subject of several somewhat inconclusive investigations on the continent, is still being investigated at Kew and the Potterne Biological Station, will not be discussed here as the work is not yet complete. Fairly large quantities of viable seed from various original sources have been kindly supplied from Potterne by Mr. Marsden-Jones. A number of non-metallic thimbles, perforated at their base by means of a bradawl, were used as minute flower-pots in each of which two seeds were sown. After attaching a piece of copper wire to each of the thimbles, they were embedded in pots of sterilized soil in such a way that the ends of the pieces of wire projected above the level of the soil. It was thus a simple matter by pulling the wires to remove a thimble and its contents

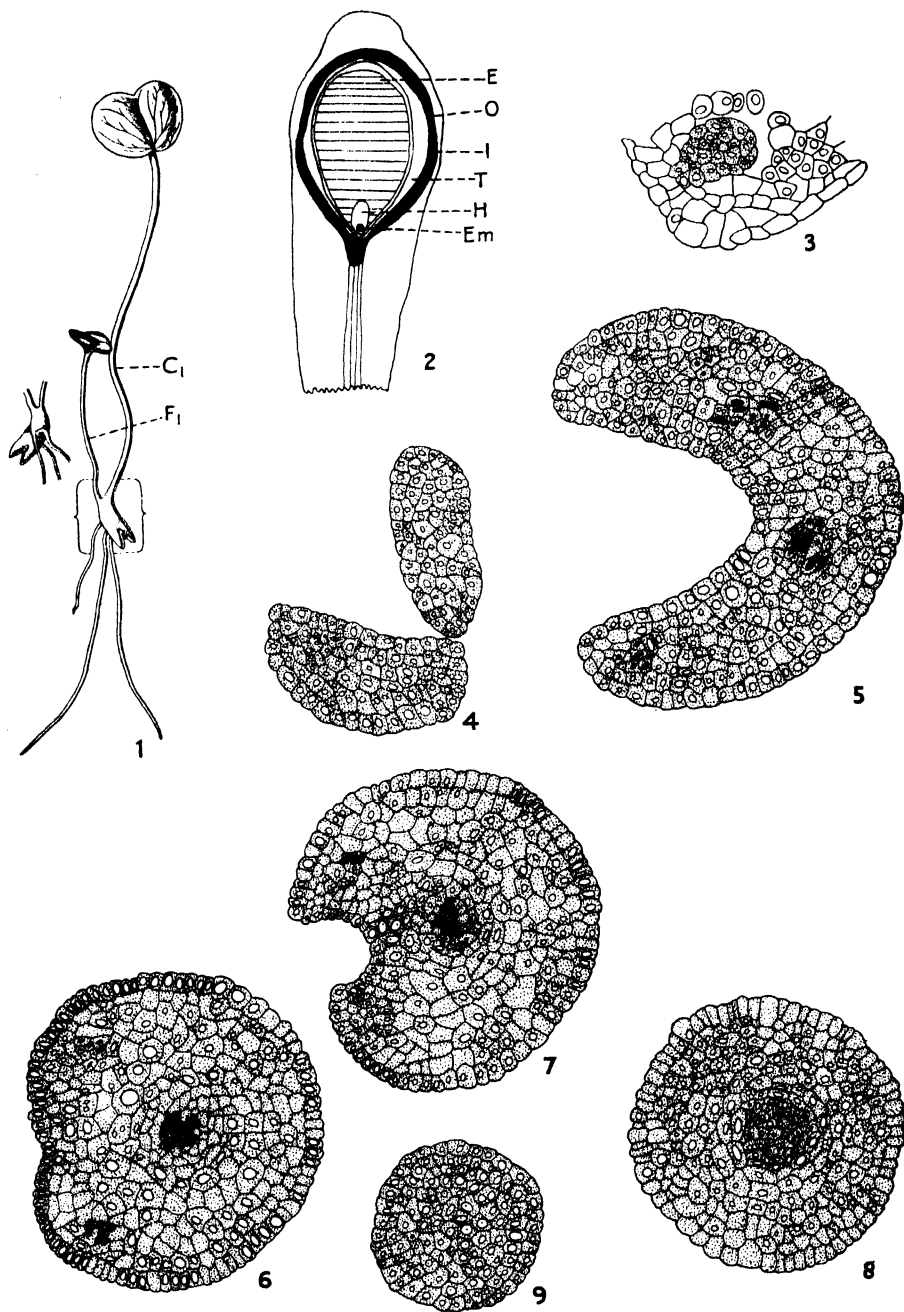
from the soil at appropriate intervals, and, having washed the seeds, to fix and embed them in the usual way. Some of the pots containing the thimbles were sunk in an open bed on a heavy clay soil at Ruislip, Middlesex, whilst others were grown in a cool pit or greenhouse at Kew. Young fruits and seeds were fixed whole in Carnoy's fluid or in formalin-acetic alcohol, but when examining older seeds the testas were removed before the seeds were fixed. After fixation the material was embedded in paraffin wax in the usual way, chloroform being used as a solvent for the wax. Sections were cut with a rotary microtome and stained in iron-alum haematoxylin. In most instances safranin was used as a counter stain. Small seedlings were embedded whole and sectioned in the same way, but older ones were cut into pieces before they were embedded.

#### EMBRYOLOGY.

The most complete account of the development of the embryo that has previously been published is the one by Sterckx, to which reference has already been made. In the main my observations agree with his. The earliest stage figured by Sterckx shows a small undifferentiated embryo at the base of a mature seed, where it is embedded in a mass of endosperm tissue. He states that the embryo began to develop two months after the seeds were sown (in May 1896), when it had the appearance of a small spherical body with a slight groove on one side extending from the apex to about two-thirds down the embryo. The remains of the small basal suspensor was still visible at this stage. After four months the embryo had elongated and the lateral groove was more clearly defined. The embryo remained dormant at this stage of development throughout the winter, developed very slightly during the following summer, and passed through a second winter before the seedling came above ground two years after the seed was sown. Very rarely seedlings came up one year after the seeds were sown. The apical growing point was found to be situated at the base of the lateral groove. It is remarkable that Sterckx's seedlings were so long in developing compared with the ones raised at Kew.

Wolter as recently as 1933, in one of the series of papers (13) published under Winkler's direction, gave an account of the development of the embryo from the time of fertilization onwards, but unfortunately his paper is not illustrated. The earliest stage observed after fertilization was a 3-celled embryo, of which the uppermost cell developed into the true embryo, the middle one into an 'Abhangsmassiv' and the basal one into a suspensor. Cell divisions proceeded irregularly, but the octants usually developed from the quadrants by vertical divisions. The subsequent divisions of the octants were so irregular that it was not possible to follow the later stages of segmentation in detail. Nor did he observe any differentiation into dermatogen, periblem, and plerome at the time when the fruit





FIGS. 1-9. Fig. 1. Typical seedling of *Ranunculus Ficaria* showing the bilobed cotyledon C<sub>1</sub> and first foliage leaf F<sub>1</sub>. The part marked by a bracket was embedded for section cutting. Fig. 2. Longitudinal section of a mature achene of *R. Ficaria* from a plant that produced viable seed. E. Endosperm. Em. Embryo. H. Hollow space. I. Tissue of thick-walled cells forming the hard wall of the mature fruit. T. Thin-walled inner portion of fruit wall.  $\times 31$ . Fig. 3. Embryo

fell. Later on the upper part of the embryo developed more rapidly than the remainder, forming a mass of tissue which was concave on one side and kidney-shaped in transverse sections. This tissue subsequently became the single cotyledon. After this the region of maximum growth became shifted to the base of the cotyledon. The growing point arose laterally to the cotyledon.

The present account of the development of the embryo of *R. Ficaria* deals only with the structure of the embryo within the mature seed and the changes that take place after the seed has been sown. Earlier stages than this are still being investigated.

Fig. 2 represents diagrammatically a longitudinal section through the centre of a typical mature achene from a plant which produced viable seed. The wall of the fruit consists of a parenchymatous outer portion (O) within which is a tissue of thick-walled cells (I), shown black in the diagram, which constitutes the hard wall of the mature fruit. Within this a narrow tissue of thin-walled cells (T) surrounds the solid mass of endosperm tissue (E), in which the food reserves are chiefly in the form of oil-drops within the cells. Situated at the basal end of the endosperm there is a hollow sphere (H), within which is the very small undifferentiated embryo (Em). The presence of disorganized cells within the otherwise hollow sphere indicates that the hollow is probably formed by the disorganization of the endosperm cells caused by enzymes secreted by the embryo itself. No stages earlier than that in Fig. 2 were examined during the present investigation. Fig. 3 shows the embryo of Fig. 2 more highly magnified. It consists of a club-shaped mass of undifferentiated parenchyma cells with prominent nuclei and dense protoplasmic contents. A few of the semi-disorganized endosperm cells near the embryo are also indicated.

In order to understand the changes which take place from this stage onwards it is necessary to realize that the embryo develops in such a way that it has but one longitudinal plane of symmetry. It thus follows that longitudinal series of sections taken at right angles to one another have quite a different appearance. In actual practice it is very difficult to obtain ideal sections illustrating this, because, during its development, the embryo frequently becomes somewhat curved within the seed. The slides from which the accompanying diagrams were made were selected from amongst a very considerable number. Drawings 3-19 are reproduced at the same

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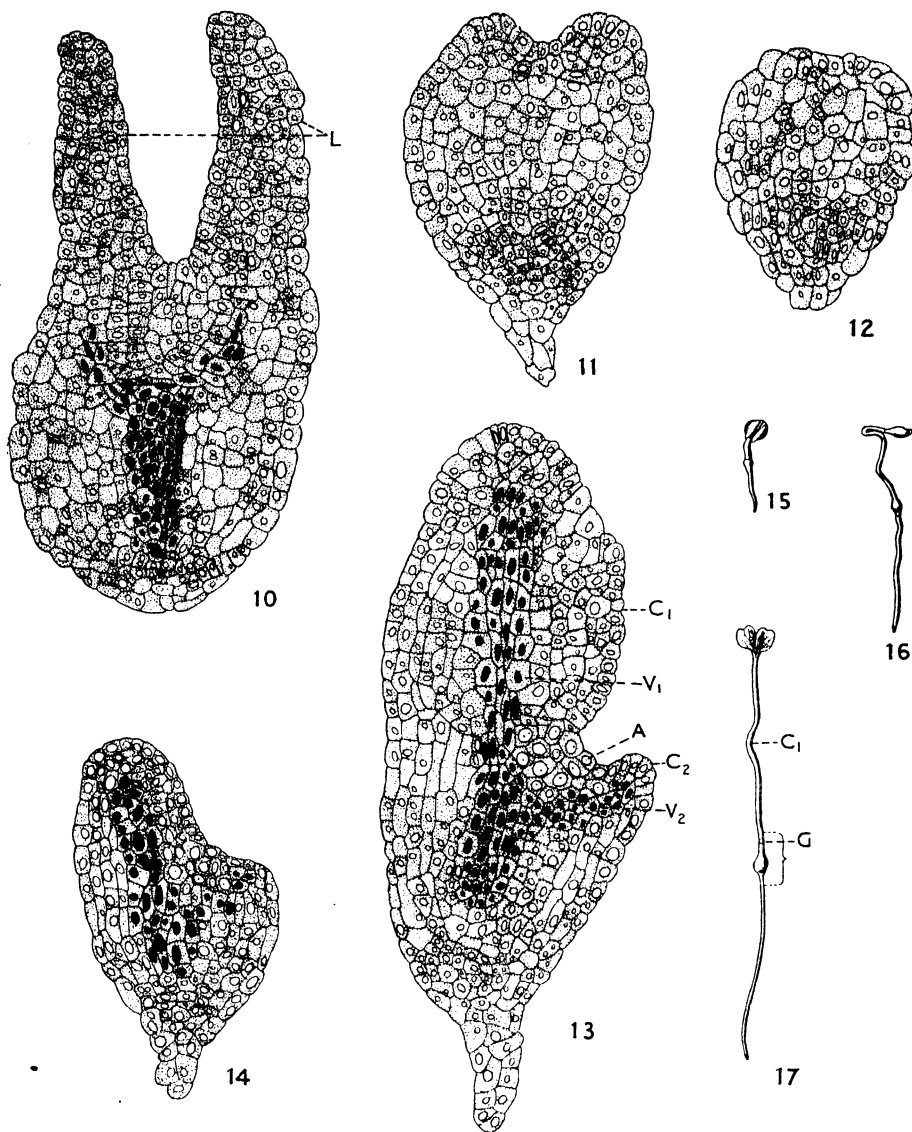
(dotted) of Fig. 2 more highly magnified.  $\times 133$ . Figs. 4-9. Transverse sections of a single embryo three months after sowing, taken at successively lower levels.  $\times 133$ . Fig. 4. T.S. apex of embryo showing the tips of the two lobes of the cotyledonary lamina. Fig. 5. T.S. embryonic lamina of cotyledon slightly below Fig. 4. Provascular bundles in black. Fig. 6. T.S. embryonic petiole of cotyledon immediately below the upper groove. Three provascular strands marked black. Fig. 7. T.S. embryo passing through the grooved base of the cotyledonary petiole. Fig. 8. T.S. embryo below the grooved base of the cotyledonary petiole. Fig. 9. T.S. embryo near the extreme base.

magnification ( $\times 133$ ), thus demonstrating how much the embryo increases in size.

The upper portion of the embryo is the first to develop. This becomes slightly indented at the apex, concave on one side, and convex on the other, thus appearing somewhat kidney-shaped as seen in transverse section. The concavity narrows progressively, and becomes shallower, as seen in transverse sections taken successively nearer the base of the embryo. At the base of the concavity, which is about two-thirds of the way down, the embryo is circular in transverse section, and below this it gradually tapers to the point at which it is attached to the surrounding tissues by means of the suspensor. Later on the apical concave end of the embryo becomes still further enlarged, and gives rise to the two lobes of the cotyledonary lamina, which are at first folded together so that the tissues which subsequently become the upper surface of the two lobes are in contact with one another. The region of maximum growth is afterwards situated lower down in the embryo so that the embryonic lamina becomes raised by the rapidly elongating petiole. The upper portion of the petiole remains grooved even when it is mature, but it is only slightly flattened or circular in transverse section throughout the greater part of its length. A second concavity at the lower end of the petiole represents the base of the original lateral concavity present in younger embryos. In other words, the original lateral concavity becomes separated into an upper and lower portion by the development of an intervening cylindrical part of the petiole. Certain cells with especially large nuclei and very prominent nucleoli develop near the base of the lateral concavity at an early stage. These mark the position in which the apical growing point subsequently arises, laterally to the cotyledon, and surrounded by its sheathing base. The most interesting fact of all, however, is that a small hump of tissue develops opposite the cotyledon, in the position in which the second cotyledon would be expected to arise if one were present. This small hump of tissue, in association with which a provascular strand arises, never enlarges. Its position and mode of origin suggest that it is the rudiment of the second cotyledon which fails to develop. This course of events will be made clearer by referring to Figs. 4-14. All these diagrams were made before the growth in the length of the embryo had begun. This takes place very rapidly, and no stages were actually observed between those in Fig. 13 and one such as that in Fig. 15, in which the radicle is seen to be emerging from the seed. The anatomy and morphology of seedlings at this and successively older stages will now be described.

#### ANATOMY AND MORPHOLOGY OF THE SEEDLING.

The external morphology of the seedling at successive stages of development is illustrated in Figs. 1, 15, 16, 17, but as this has been fully



FIGS. 10-17. Figs. 10-12. A series of longitudinal sections of a single embryo of *R. Ficaria* two months after sowing.  $\times 133$ . Fig. 10. L. Lobes of the cotyledonary lamina. Provascular strands marked black. Fig. 11. L.S. embryo passing through the bases of the two lobes of the cotyledon which appear as two terminal humps. Fig. 12. L.S. embryo on the side away from the cotyledon. Fig. 13. L.S. embryo of *R. Ficaria* three months after sowing, cut in a plane at right angles to those in Figs. 10-12. A. Position in which the stem apex arises. C<sub>1</sub>. Cotyledon. C<sub>2</sub>. Rudiment of second cotyledon. V<sub>1</sub>. Provascular strand to cotyledon. V<sub>2</sub>. Provascular strand to rudiment of second cotyledon. Fig. 14. L.S. embryo of *R. Ficaria* in the same plane as that in Fig. 13 but of a younger embryo (one month after sowing).  $\times 133$ . Figs. 15-17. Three stages in the development of the seedling of *R. Ficaria*. In Fig. 17 the cotyledon tends to be trilobed. G. Position of the stem apex. The part of the seedling marked by a bracket was embedded for section cutting.

described by some of the workers already referred to, it is unnecessary to give further details. Figs. 18–24 represent transverse sections at successively lower levels through a seedling such as that of Fig. 15. In Fig. 18 the mass of endosperm tissue (E) can be seen enclosing the two terminal lobes of the cotyledonary lamina (C) which are situated loosely in a hollow space in the centre of the endosperm. The numerous provascular bundles (v) are shown in black. At this stage they are not fully differentiated into phloem and xylem. At a lower level (Fig. 19) the two lobes of the cotyledon are united at the margin, and anastomoses between the provascular bundles occur. In Fig. 20 the grooved upper part of the cotyledonary petiole is shown with one relatively large provascular strand at the centre and the two smaller ones on the same side as the groove. This characteristic arrangement is maintained in a majority of cotyledons throughout their length. Cotyledonary petioles with only one central strand also occur, although they have not been met with so frequently. The structure of the seedling at levels below that in Fig. 20 can best be understood by referring to Figs. 21–24, which are self explanatory.

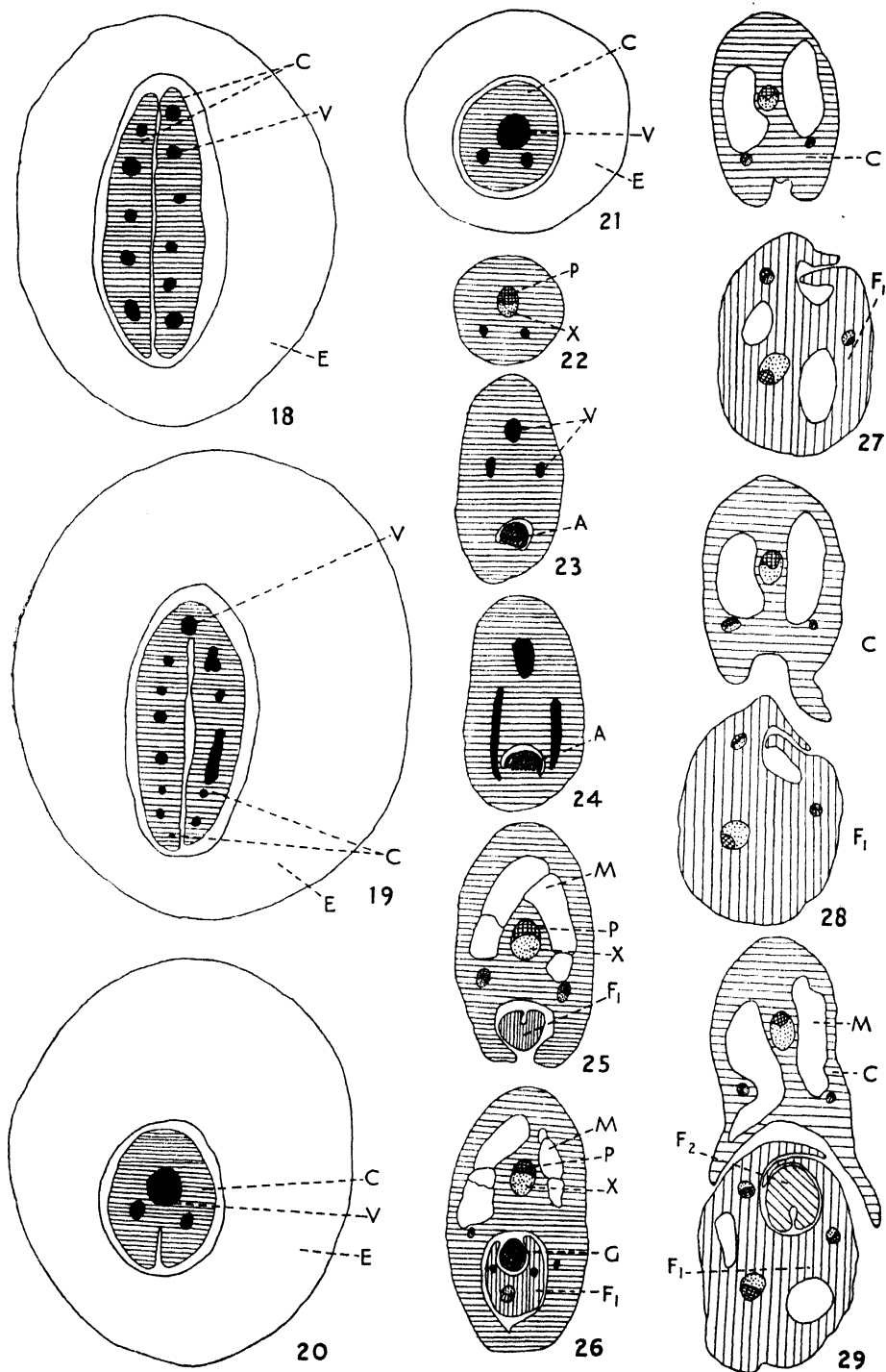
Transverse sections of older seedlings such as those in Figs. 1 and 17 were cut only in the region of the growing point, those parts of the seedlings marked with brackets having been cut out and embedded. Transverse sections of a seedling such as that in Fig. 17 are illustrated in Figs. 25 and 26. In Fig. 25 the winged margins at the base of the cotyledon can be seen partly enclosing the grooved first foliage leaf (F1). The three vascular bundles of the cotyledon are completely differentiated and consist of collateral groups of xylem and phloem. Large mucilage cavities (M), which are very frequent in *R. Ficaria*, are also indicated. In Fig. 26 the sheathing base of the cotyledon is surrounding the grooved first foliage leaf, which, like the cotyledon, is provided with one large central and two smaller lateral vascular bundles. The mass of undifferentiated tissue (G), from which subsequent foliage leaves will arise, is partly enclosed within the groove of the first foliage leaf.

Transverse sections through and near the growing-point region of a seedling at the stage in Fig. 1 are illustrated in Figs. 27–33. The sections in Figs. 27 and 28 pass through the bases of the cotyledon (C), and the first foliage leaf (F1). The most important features to note are (1) that the cotyledonary and first foliar petioles are grooved and (2) the cotyledon and first foliage leaf have the same type of vascular anatomy, one large central and two smaller lateral bundles being present in both these organs. Figs. 29, 30, and 31, which represent sections taken at successively lower levels in the seedling, indicate how the second, third, and fourth foliage leaves (F2, F3, F4) are successively developed round the growing point (A). All these foliage leaves have essentially the same vascular structure as that of the cotyledon. The first and second foliage leaves are provided with the

three characteristic vascular bundles, but the seedling from which the sections were taken was not old enough to show the full number of bundles in the third and fourth leaves, only the central provascular bundles being visible in the third foliage leaf and none in the fourth (Fig. 31). Another feature indicated in Figs. 22-31 is that the margins of the sheathing base of the first foliage leaf approach one another so closely that the growing point and embryonic leaves which develop subsequently to the first are enclosed within it. At still lower levels in the same seedling (Fig. 33) the sheathing cotyledon entirely surrounds the growing point, so that all the leaves are enclosed within it. In Fig. 32 the free end of a bud (B) in the axil of the cotyledon is shown. In the region shown in Figs. 32 and 33 there are large masses of embryonic tissue (circles) within which (Fig. 33) two smaller bundles of the first foliage leaf lose their individuality.

Before attempting to draw conclusions concerning the morphology of the cotyledon in the light of these facts, brief notes on the anatomy of the dicotylous *R. arvensis* and *R. muricatus* are given. Figs. 34-37 represent transverse sections taken at successively lower levels through the base of the cotyledons and the region immediately above the growing point of a seedling of *R. arvensis*. Fig. 34 shows the two opposed cotyledons each with three collateral vascular bundles, and the folded apex of the first foliage leaf with six bundles. In sections at this level the two cotyledons are entirely free from one another, but slightly lower down (Fig. 35) they are united at one margin by a rather definite mass of fusion tissue (T). In Fig. 36 the two cotyledons are merged together completely at one margin, the line of union being indicated only by a small mass of fusion tissue on the inner wall of a large mucilage cavity (M). In this region there is a large mass of fusion tissue where the two cotyledons are united by their margins on the opposite side of the seedling as well (T<sub>2</sub>) so that the base of the two cotyledons forms a tube within which the foliage leaves develop. Fig. 36 indicates that the three bundles of each of the cotyledons are still visible at this level. There are three collateral bundles in the first foliage leaf, but none are visible in the second one at this level. At the level illustrated in Fig. 37 the bases of the two cotyledons form a very definite tube, the marginal regions being represented only by two small masses of fusion tissue (T<sub>1</sub> and T<sub>2</sub>). The three bundles of each of the cotyledons have joined together to form one large solitary bundle. The first foliage leaf has three fully differentiated bundles, but in the second there are one fully differentiated and two provascular bundles, three provascular ones in the third and in the fourth no vascular tissue has been differentiated.

A similar series of sections through a seedling of *R. muricatus* exhibits essentially the same structure, and only one section in the region of the stem apex is illustrated (Fig. 38). The bases of the two cotyledons form



a tube, and there is a single large bundle in each of the united cotyledons. Three main bundles are being formed in each of the foliage leaves.

In comparing the seedlings of *R. arvensis* and *R. muricatus* with those of *R. Ficaria* the orientation of the cotyledons in relation to the foliage leaves is worthy of note. In *R. Ficaria* the first foliage leaf arises opposite to, but included within, the sheathing base of the single cotyledon, while in the other species of *Ranunculus* the two cotyledons are opposed but united at their bases by the margins. The first foliage leaf is orientated approximately at right angles to the two cotyledons.

#### CONCLUSIONS.

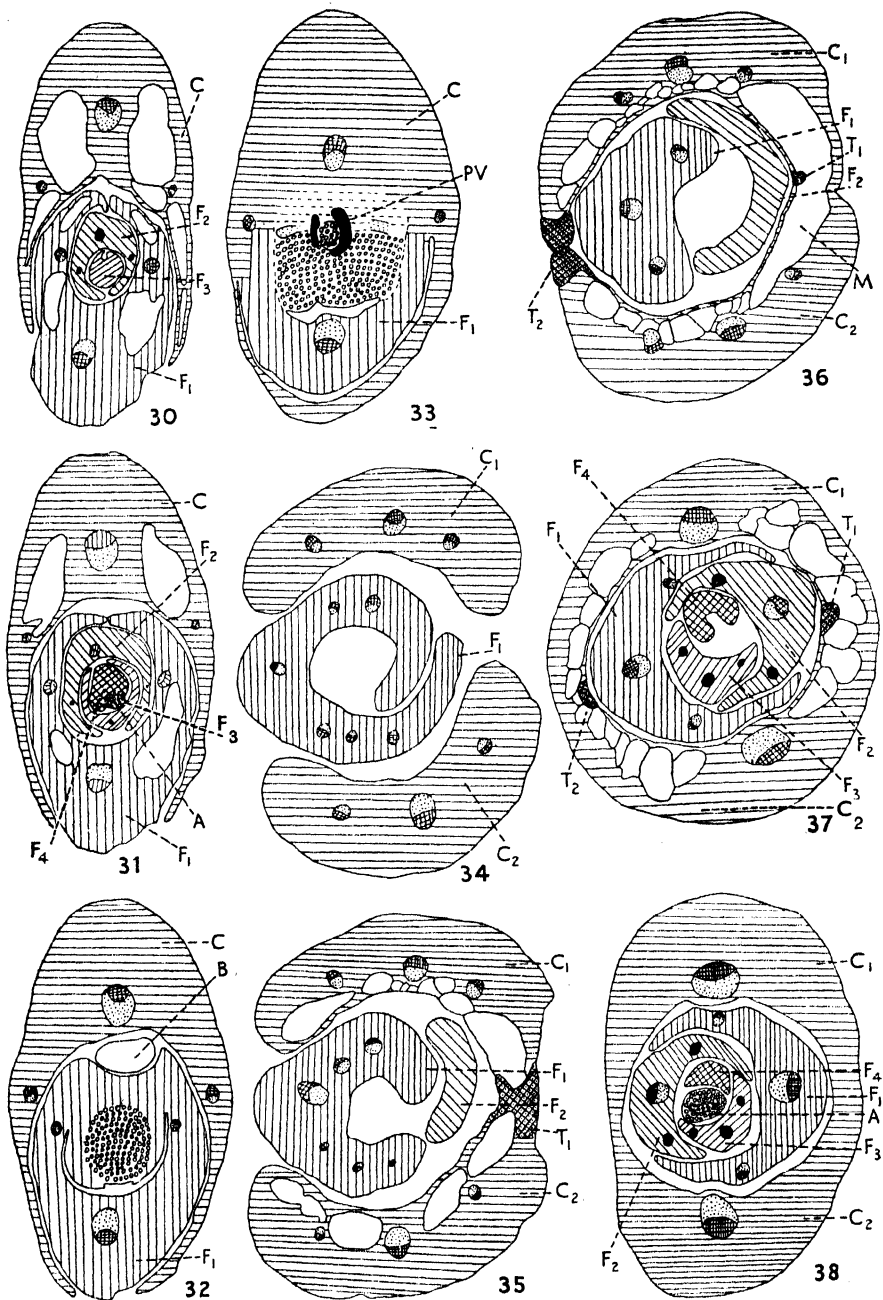
The external morphology and anatomy of the seedling as well as the late embryology of *R. Ficaria* are believed to demonstrate quite definitely that this species has become monocotylous by the suppression of one cotyledon. This conclusion is of considerable phylogenetic interest, more especially in view of the suggestion that there are affinities between the Ranales and the true Monocotyledons.

Those who hold that the cotyledon of *R. Ficaria* is syncotylous point out that this species alone amongst the Ranunculaceae is known normally to have a cotyledon with a bifid lamina. Moreover, Miss Sargent has asserted that there is no main vein between the top of the cotyledonary petiole and the base of the apical indentation, such as would be expected to be present if the cotyledon is a single organ. In addition it has been claimed that the groove in the upper part of the cotyledonary petiole is a relic of the hypothetical line of fusion between the two ancestral cotyledons. It is certainly remarkable that *R. Ficaria* should differ from other Ranunculaceae in having a bifid cotyledon, but it would seem to be of no special significance since bifid cotyledons are present in so many other Dicotyledonous families. Moreover, each of the two cotyledons on H. Winkler's abnormal seedlings is bifurcated in the same way as in the normal single one. Then again the occurrence of trilobed cotyledons may

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FIGS. 18-29. Figs. 18-24. Transverse sections at successively lower levels of a single seedling such as that in Fig. 15.  $\times 31$ . Fig. 18. C. Free lobes of cotyledon. E. Endosperm. V. Provascular bundles marked black. Fig. 19. C. Lobes of cotyledon united at the midrib. E. Endosperm. V. Provascular bundles. Fig. 20. C. Grooved upper end of cotyledonary petiole. E. Endosperm. V. Provascular bundles. Fig. 21. C. T.S. cylindrical portion of cotyledonary petiole. E. Endosperm. V. Provascular bundles. Fig. 22. T.S. cotyledonary petiole below the endosperm. The central vascular strand is differentiated into phloem P and xylem X. The two smaller bundles are not yet fully differentiated. Fig. 23. T.S. sheathing base of cotyledonary petiole with three provascular strands V surrounding the tissues near the stem apex A. Fig. 24. T.S. sheathing base of cotyledon with three provascular bundles passing towards the stem apex A. Figs. 25-26. Transverse sections at successively lower levels of a seedling as in Fig. 17, passing near the growing point. G. Growing point (stem apex). F1. First foliage leaf. M. Mucilage cavity. P. Phloem. X. Xylem.  $\times 31$ . Figs. 27-29. Transverse sections at successively lower levels in the growing-point region of a seedling as in Fig. 1. (This series is continued in Figs. 30-33.) C. Cotyledon. F1-F4. First four foliage leaves. M. Mucilage cavity.





FIGS. 30-38. Figs. 30-33. Transverse sections at successively lower levels in the growing-point region of a seedling as in Fig. 1. (This series is continued from Figs. 27-29.) A. Stem apex. C. Cotyledon. F<sub>1</sub>-F<sub>4</sub> First four foliage leaves. M. Mucilage cavity. B. Bud in the axil of the cotyledon. Embryonic tissue marked with circles. Provascular tissue black.  $\times 31$ . Figs. 34-37.

be taken as additional evidence that the two lobes of the lamina are of no phylogenetic significance.

Little importance can be attached to Miss Sargent's claim that the 'opening out' of the single bundle at its base is evidence that this bundle is morphologically equivalent to two fused together, firstly, because it is common to find cotyledonary petioles with three bundles as in the young foliage leaves, and secondly, because, in spite of the 'opening out' of the bundle to be observed in Miss Sargent's slides, the bundle is evidently a single one surrounded by one sheath. There is no evidence of a true separation into two bundles. The three bundles present in each of the cotyledonary petioles of the dicotylous *R. arvensis* may be taken as evidence that each cotyledonary member is homologous with the single one in *R. Ficaria*. In the limited material of *R. muricatus* that has been examined, each cotyledonary petiole has only one bundle as in the case of some specimens of *R. Ficaria*. In the petioles of old foliage leaves of *R. Ficaria* there are frequently more than three bundles, the additional ones being formed near the margins as the leaves grow older. The three bundles are, however, quite characteristic of all the young foliage leaves that have been examined. Thus the evidence provided by the vascular anatomy suggests that the single cotyledon is equivalent to, but not homologous with, a reduced foliage leaf, which, it may be presumed, has become modified in correlation with its special function of absorbing nourishment from the endosperm.

Having thus arrived at the conclusion that the single cotyledon is equivalent to a single foliar organ, it is especially interesting in following the development of the embryo to have discovered that a hump of tissue develops in the position in which the second cotyledon would be expected to arise if one were present. That this hump is a potential cotyledon which fails to develop is highly probable. H. Winkler's record of the occurrence of seedlings of *R. Ficaria* with two cotyledons leads naturally to the suggestion that in rare instances the hump may still develop into the second cotyledon in a way that we may presume was at one time normal for the species. It is also interesting to note that the embryo of *R. Ficaria* in some respects resembles those of certain Alismataceae. Schaffner (8) in 1897 wrote an illustrated account of the embryology of *Sagittaria variabilis*, and in Fig. 83 of his paper, which represents a longitudinal section through the growing point of a mature embryo, there is a hump of tissue opposite the cotyledon from which it is separated by the

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Transverse sections of a seedling of *R. arvensis* at successively lower levels near the growing point. C1 and C2. Cotyledons. F1-F4. Successive foliage leaves. T1 and T2. Two groups of fusion tissue situated where the margins of the cotyledonary petioles are joined together.  $\times 31$ . Fig 38. T.S. seedling of *R. muricatus* in the growing-point region. A. Stem apex. C1 and C2. Cotyledons. F1-F4. Successive foliage leaves.

stem apex as in my Fig. 13 of *R. Ficaria*. It is true that Schaffner believed this hump, of which he does not appear to have followed the subsequent development, to be the young first foliage leaf. A more likely interpretation seems to be that, as in *R. Ficaria*, it represents an abortive second cotyledon. If it were a foliage leaf it would be expected to arise at a higher level on the axis than the single cotyledon to which it is opposed. Further work on the *late* stages in the embryology of the Alismataceae is evidently needed to clear up the homology of this parenchymatous hump.

The seedling of *R. Ficaria* is also in some respects comparable with that of *Cyclamen*. Sir Arthur Hill (4) has shown that in this genus of Dicotyledons the seedling normally has but one cotyledon. The second one, which is abortive, may, however, be induced to develop by performing surgical operations on the single cotyledon.

Sir Arthur Hill's work on *Peperomia* seedlings (2) is also interesting in this connexion. He has shown that in certain geophilous species of this genus the seedlings have become heterocotylous. One of the cotyledons is a green, aerial, assimilating organ, and the other remains embedded in the seed when it germinates and serves primarily for the absorption of food from the reserves in the seed. Comparisons are drawn between those *Peperomias* whose cotyledons exhibit this division of labour and certain true Monocotyledons in which the cotyledon absorbs food from the reserves in the seed, and the suggestion is made that the apparent first foliage leaf of Monocotyledons, which is a green assimilating organ, may be homologous with the second cotyledon. In a later paper (3) reference is made to an abnormal seedling of *Arisarum vulgare* Targ.-Tozz. The seedling of this species normally has a cotyledon which remains embedded in the seed and serves primarily for the absorption of food. From the abnormal seedling this cotyledon was absent, and its function was carried out by the organ which is normally regarded as the first foliage leaf. This state of affairs is the converse of that in the geophilous *Peperomias*, but it tends to support the suggestion that the apparent first foliage leaf in certain Monocotyledons may be homologous with the second cotyledon. From these examples it seems possible that the monocotylous habit may have been adopted more than once in the course of evolution, and that the change took place along different lines in the several instances. Whether on any of these occasions the monocotylous habit was effected by the fusion of two original cotyledons is still uncertain, but, as additional information becomes available, it seems progressively more improbable that this change ever took place.

SUMMARY.

1. The external morphology, seedling anatomy, and late stages in the embryology of *R. Ficaria* are described. Evidence taken from these facts is thought to show conclusively that the single bifid cotyledon, which is normally present in this species, represents a single foliar organ and not two fused together as has previously been held by some botanists.

2. The venation of the cotyledon, which normally has a well-developed median vein extending to the base of the apical indentation, is consistent with the view that the cotyledon is a single foliar member.

3. The presence of a longitudinal groove at the upper end of the cotyledonary petiole cannot be regarded as a relic of the hypothetical line of fusion between two cotyledons because similar grooves are present in the foliage leaves.

4. The rare occurrence of *R. Ficaria* seedlings with two bifid cotyledons recorded by Winkler does not favour the suggestion that each lobe of the normal single bifid cotyledon represents the lamina of a single ancestral cotyledon.

5. Trilobed cotyledons sometimes occur, the existence of which does not favour the syncotylous theory.

6. The cotyledonary petiole contains either a singular vascular strand throughout its length or a large median bundle with two small lateral ones. The petioles of young foliage leaves, and each of the cotyledonary petioles of *R. arvensis*, are provided with three bundles in the same way. The few cotyledonary petioles of *R. muricatus* that have been examined show only one vascular strand as in some of the cotyledonary petioles of *R. Ficaria*. These facts are thought to show that the cotyledon of *R. Ficaria* is comparable with a single foliage leaf and homologous with a single cotyledon of other species of *Ranunculus* which normally have two, but that it shows a tendency for the vascular system to be reduced.

7. The embryo in a mature seed of *R. Ficaria* is a small undifferentiated body, shaped like the head of a club, situated in a hollow sphere at the base of the seed surrounded by endosperm tissue.

8. During its slow development the embryo becomes zygomorphic, and, for a period, the greater part of it consists of a mass of tissue which is crescent-shaped in transverse sections. This is the embryonic single cotyledon. Later on the cotyledonary petiole elongates very rapidly.

9. The growing point arises laterally to, but embedded in, the base of the cotyledon.

10. A small parenchymatous hump, which is supplied with a provascular strand, is formed in the position in which the second cotyledon would be expected if one were present. This hump, which does not normally

develop further, is thought to represent the rudiment of the second cotyledon.

11. The morphology of the single cotyledon is of considerable phylogenetic interest because of the generally assumed close connexion between the Ranales and the true Monocotyledons. Comparisons are also drawn between the seedlings of *R. Ficaria* and those of *Cyclamen* in which genus one cotyledon is suppressed, and certain species of *Peperomia* which are heterocotylous.

Amongst all those who have assisted in various ways with this investigation, I am especially indebted to Sir Arthur Hill at whose suggestion the work was undertaken, and whose interest and encouragement have been of the very greatest help. Thanks are also due to Dr. W. B. Turrill who examined many of my slides and made many helpful suggestions. The greater part of the material was kindly supplied from the Potterne Biological Station by Mr. E. M. Marsden-Jones, whose collaboration in this way has been of very great assistance. Thanks are also due to the authorities at Girton College, Cambridge, for the loan of Miss Sargent's slides, and to Miss M. W. Tanner for drawing Figures 1 and 15-17.

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# The Production of Root-hairs in Relation to the Development of the Piliferous Layer.<sup>1</sup>

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With twelve Figures in the Text.

THE connexion between the conditions and rate of growth of the root as a whole, and the production of root-hairs, has received some attention in the past. The results of observation are conflicting. The development of root-hairs has been variously described as being due to (or at least an accompaniment of) a retardation of growth of the root, and as a concomitant of optimum conditions and maximum growth rate. The former view appears to have been more generally accepted. Some of the evidence on which it is based appears, however, to be questionable, and there is good evidence that the second view is more correct.

In any case, the criterion of root-hair development used, i.e. the frequency of the hairs expressed as the number per unit area, seems to be unsatisfactory. It is proposed to bring forward evidence that the production of root-hairs is an accompaniment of good growth conditions and to suggest another criterion of root-hair development by the use of which some of the discordant views of various workers may be brought into line.

Mer (10) and (11) regarded hair production as a lateral outgrowth stimulated by retardation of growth of the root-tip. He noted that when lentil and other seeds were germinated on the surface of damp soil they did not produce hairs immediately—there was a glabrous region immediately below the cotyledons. Hair production was stated to occur when the root-tip made contact with the soil surface. The roots were often swollen in their hairy regions, and when these parts were curved the hairs were more numerous on the convex side. Mer also germinated seeds on corks floating on water and observed that when the root met the water surface there was a more abundant hair production, which was not sustained. This was explained by assuming that the change of medium causes retardation of growth and production of hairs; the root then accommodates itself to the new medium, the growth rate again increases, and root-hair production falls off as the stimulus causing it has ceased to operate.

<sup>1</sup> Thesis approved for the Degree of Master of Science in the University of London.

The production of hairs by retardation of growth was supposed to be due to a diversion of food materials from the axis to the lateral growth centres, including the epidermis, which is thus enabled to produce hairs.

Mer's conclusions are, however, to a certain extent contradictory. He notes, for instance, that hair production is more prolific on the convex side of a curved root, a region which will have a higher growth rate than the less hairy concave side where the retardation of growth occurs. His conclusion that exhaustion of the cotyledons may cause hair production (by causing growth retardation) is also at variance with his postulate that hair production is due to an extra food-supply to the epidermis. He also appears to be wrong in attributing initiation of root-hair formation in *Lens* to mechanical retardation of the root-tip by contact with the soil. Root-hairs will appear in the absence of the contact, and further, however early the contact takes place there is always a glabrous region immediately below the cotyledons. This, in part, is the hypocotyl and cannot thus be expected to produce root-hairs. Mechanically perfect stomata are present in this region. The remainder of the glabrous zone probably represents an elongation of the radicle laid down in the seed.

The gradual cessation of hair formation in *Lens* that Mer observed, when seedlings were allowed to grow from air into water, is also open to a more satisfactory explanation than that offered by Mer. If the water is kept well aerated there is very little change in root-hair development over the immersed regions, though there may be an apparent transitory increase in hair formation when the root meets the water surface.

Snow (17) also regarded retardation of growth as an important factor in causing hair production. Thus, *Elodea* roots developed in water were hairless, but became well provided with hairs on entering a substrate of soil or quartz. At the same time the average epidermal cell length was reduced. This retardation was regarded as being mechanical or chemical in nature. Other experiments, however, with maize showed slower growth in water than in air, and an associated smaller hair development. An attempt was made to induce hair formation by mechanical retardation of the root with a glass rod. The results of these experiments were, however, unsatisfactory as hairs sometimes appeared without the contact. Experiments on the retardation effects of different media also appear to be unsatisfactory in so far as the media used varied in compactness and water-holding capacity. Removal of the cotyledons of seedlings to varying degrees reduced hair formation, hairs generally being most prolific when the food-supply was most abundant. Lack of oxygen was also reported as reducing hair production. Curves and swellings had a favourable effect on hair formation. Snow attributed this to their representing the retardation of growth of the root. Where retardation of growth was held to favour hair development it was stated that the mere rate of growth was not important,

but that the differential elongation of the inner and outer cells was the significant factor. Root-hair production was held to be dependent on the ratio between the capacity of the epidermal cells to elongate and their ability to do so as limited by the slower-growing sub-epidermal tissues. The epidermal cell being restrained from elongation in the direction of the axis produces a lateral outgrowth in the shape of a hair. By measurements of the lengths of epidermal cells and the underlying cortical cells Roberts (13) showed that this supposed relationship between the dimensions of these cells cannot be substantiated. Snow's conclusion that lack of oxygen may cause a reduction in root-hair development is supported by Cannon (1). The last-mentioned paper is concerned almost entirely with root growth in different oxygen pressures, but a few notes were made on root-hair development.

Masters (9) concluded from germination experiments that there is no correlation between hair production and root length, and that the production of hairs caused apparently by an obstacle is probably to be explained by the stimulating effect of the water-films with which the obstacle would most probably be covered.

Schwarz (15) considered that root-hair formation is not induced by retardation of growth, but is an accompaniment of maximum growth conditions. He was not able to cause hair formation by arresting growth with wire gratings, nor with glass tubes, though, as Snow pointed out, unlooked-for toxic effects may possibly have been operating. Schwarz did not consider the resistance of the earth to be a factor causing hair formation, though he found that it might induce hair formation to begin at an earlier stage, the hairs appearing nearer the root-tip. Snow comments on this as really being a favourable effect, in that the hairs elongate in a region where they otherwise would not do so. The results of Schwarz are probably explicable on the assumption that the epidermal cells reach maturity closer to the tip as they do not grow so large. Hairs may thus appear earlier. In this connexion it is interesting to note that chemical retardation may have the same effect. Experiments carried out by the writer on *Lens* show that roots grown in air containing 10 per cent.  $\text{CO}_2$  are slower growing and produce their root-hairs nearer the tip than control roots grown in air. Hairs appear 2-3 mm. and 8-15 mm. respectively from the root-apex in the two cases.

Schwarz also refuted Mer's statement that root growth is faster in water than in air and showed that under the conditions of his experiments it was fastest in damp air and progressively slower in damp soil and in water. Root-hair development bore a direct proportion to the growth rate of the root. He experimented with *Elodea*, and while agreeing that hair production occurs only on roots in soil, he was unable to induce hair formation by contact with glass beads or ground glass, and hence thought that some factor other than contact was operating. It is, of course, open to



question that the glass may have had an unfavourable effect by virtue of its alkaline nature, particularly as Snow was able to induce hair formation by the use of quartz. Schwarz's conclusions are in accordance with those of Mer and Snow in so far that he believed kinking of the root to be accompanied by increased hair production, but he regarded the kinking not as the result of growth retardation, but as evidence of maximum growth energy.

In their observations on root-hair production Snow and Roberts have made measurements of hairy and non-hairy epidermal cells in the hope that these would throw some light on the formation of hairs. It was suggested that hair formation is accompanied by a change in the dimensions of the epidermal cell bearing the hair.

These measurements were made on individual cells, and comparisons were made between nearby hairy and non-hairy cells in the same region of the root. They did not point to any definite conclusion except that they threw suspicion on Snow's suggestion that the relationship between the dimensions of the epidermal and cortical cells was of importance in determining hair formation. It would appear, however, that if any difference in size of the epidermal cell is an accompaniment of hair formation, it should be looked for, not as between hairy and non-hairy cells of the same region of the root, but as between the average dimensions of all epidermal cells, piliferous and non-piliferous, on hairy and non-hairy roots, for the connexion between adjacent cells of the epidermis is at least as close as between the epidermis and the cortex.

Observations have, therefore, been made on a number of species, and these show that in general hair formation is accompanied by an increase in the dimensions of the cells of the piliferous layer, which is mostly in the direction of elongation and sometimes also an extension in width. Most of the species used in this examination are aquatic plants. Normally the roots of such species are growing in relatively uniform media of water or mud, and hence show a fairly even development of the root. Changes in conditions and the presence of obstructions to growth, which may cause irregular development, and hence make it difficult to make representative estimates of the size of the epidermal cells and of the intensity of hair formation, are correspondingly less frequent in their occurrence than in terrestrial species. The task of removing adhering soil from the roots is also considerably lessened by the use of water-plants, and this is a serious obstacle to the examination of the piliferous layer and hair formation of plants of drier habitats.

In plants where the root system is fairly complex it is desirable to examine roots of the same order so that a more accurate comparison is obtained. In this connexion the possibility of dimorphism must not be ignored, e.g. in species of *Carex*. Schwarz refers to a similar state of affairs in *Sagittaria sagittifolia*.

*Elodea* has previously been examined by others in respect of its root-hair development, with somewhat discordant results. It is well established that hairs are normally produced only on roots which have entered the substratum. The hairs are formed from special cells differentiated quite near the root-tip, which are distinguishable from the other non-potentially hair-bearing cells of the piliferous layer even in non-hairy roots. If no contact with a substratum takes place and hairs are not formed, the potentially hair-bearing cells tend to lose their individuality and become difficult to distinguish from the others.

Quartz was sufficient to induce hair formation (Snow) and clean sand is also quite efficient in this respect, so that a contact stimulus appears to be operating. At the same time Schwarz was unable to induce hair formation by the use of ground glass or glass beads as a substrate. Measurements of the epidermal cells grown both under natural conditions and in the laboratory give results opposite to those of Snow in that the hairy roots consistently have longer epidermal cells than the non-hairy. In what way the substrate stimulates hair formation is not clear. It can hardly be a matter of aeration. It is perhaps possible that the substrate, by adsorption, makes available to the roots a higher concentration of mineral salts than is present in the solution; these would be obtained by the root by base exchange. An explanation such as this, however, presupposes a great sensitivity on the part of the roots to changes in concentration of the minerals; a sensitivity which experiments do not confirm. Coarse sand used as a substrate in tap-water will induce hair formation, but even in quarter-strength Knop's solution contact with sand is still necessary for the development of hairs. It would also appear to be significant in this respect that roots grown in full-strength Knop's solution still produced longer epidermal cells in a sand substrate than in the free solution, although the concentration of salts was obviously above the optimum and no hairs were formed at all. The change from the non-hairy to the hairy condition appears to be so closely associated with the penetration of a substrate that the only stimuli which would appear to be acting are those of contact and suddenly reduced light intensity. Schwarz found that ground glass used as a substrate was ineffective in causing hair production, which suggests that lack of illumination may be the causal factor. It is important to note, however, that the pH of water in contact with ground glass may be relatively high, and this may very well be adequate to suppress formation of hairs, particularly as the plants grown in ground glass were obviously unhappy. An experiment was carried out in which four roots of *Elodea* were induced to grow in ground glass; none of them formed hairs.

Accordingly a light tight ventilated chamber was constructed of tin-plate and enamelled (Fig. 1). This when stood in a glass tank of water with an inch depth of sand enabled sprigs of *Elodea* to be grown with their

lower parts in darkness. Eleven roots were developed in these darkened regions, being encouraged by the removal of all roots which were formed outside the dark chamber. None of the roots grown in darkness produced hairs until two penetrated the substratum, when hairs appeared on the regions affected in this way. Evidently, therefore, the production of roots is not affected by light but is bound up with a stimulus which comes into play when the root penetrates a substratum—this stimulus is probably of a tactile nature.

The frequencies of the epidermal cells and of the root-hairs developed under the various conditions under which *Elodea* has been studied are given in tabular form below. The frequencies of the epidermal cells were determined from measurements of camera lucida drawings of surface sections of the root, and the root-hair frequencies were arrived at by calculations based on the average number of hairs observed in  $100\mu$  transverse sections cut by a hand microtome. (See Fig. 2 for piliferous layer of hairy and hairless roots.)

*Elodea. Development of Epidermal Cells and Root-hairs.*

Roots in tapwater.	Cell frequency. per mm. <sup>2</sup>	Hair frequency. per mm. <sup>2</sup>
(a) in a sand substrate	211 217	45 50
(b) in the water	270 255	nil "
(c) in the water, in darkness	256 268	" "
Roots in $\frac{1}{4}$ -strength Knop's solution.		
(a) in sand substrate	158, 162, 195	36, 42
(b) in solution	216 233	nil "
Roots in full-strength Knop's solution.		
(a) in sand substrate	277 249	" "
(b) in solution	388 393 394	" " "
Roots in stream under natural conditions.		
(a) in mud	{ 204 } { 247 }	68, 56, 5
These specimens were obtained from two different sources.	{ 143 } { 120 } { 141 }	45, 50, 48
(b) in the water	296 272 258 200 292	nil " " " "

It will be noted that these results are in contradiction with those of Snow, in that she found a positive correlation between hair production and a reduction in the size of the hair-forming cells.

This discrepancy would appear to be explicable on the following

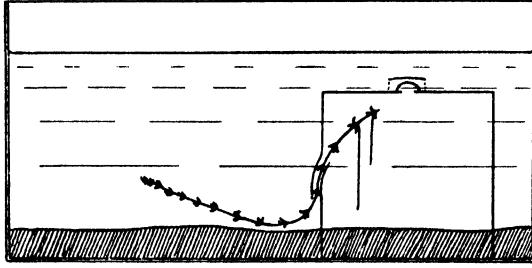


FIG. 1. Method of growing roots of *Elodea canadensis* in darkness. (Reduced.)

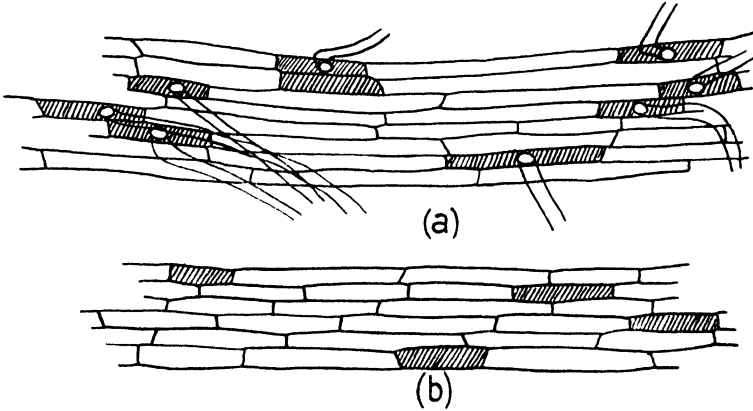


FIG. 2. *E. canadensis*; Piliferous layer of hairy and non-hairy roots grown in Knop's solution ( $\frac{1}{4}$ -strength).  $\times 87$ .

grounds. Snow made measurements of hairy and non-hairy cells in the same region of the root, while the measurements given above are based on average values of the frequencies of all the cells of hairy and non-hairy roots. The root-hairs of *Elodea* are produced from specialized hair-cells, which are distinguishable from the non-hair-producing cells near the root-tip (Fig. 3), but are not, however, in the mature regions of the root, very obvious, and it appears probable that Snow failed to recognize their true nature. As a result her measurements do not give an accurate picture of the changes which occur in the development of the cells of the piliferous layer concurrent with root-hair formation.

*Potamogeton densus* also develops its hairs from previously differentiated hair-forming cells, but, unlike *Elodea*, the outgrowth of hairs is independent of the root penetrating a solid substrate. As for *Elodea*, the cell frequency

and root-hair frequency in *P. densus* were determined from camera lucida drawings of the epidermis (Fig. 4). The root-hair frequency was also determined independently by the section method. A considerable discrepancy was observed between the values of the root-hair frequency obtained

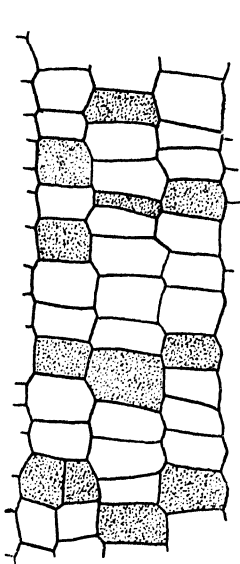


FIG. 3. *E. canadensis*; Surface view of piliferous layer 2 mm. behind root-tip (observed after removal of the root-cap), showing early differentiation of the root-hair-forming cells.  $\times 375$ .

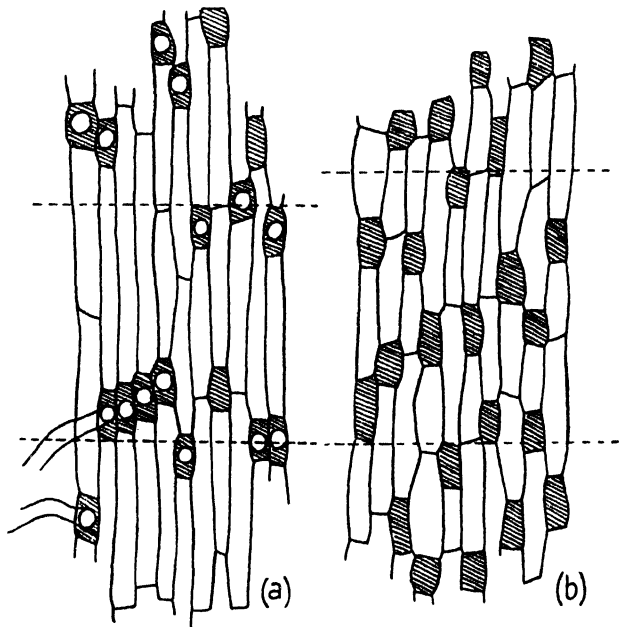


FIG. 4. *Potamogeton densus*; Piliferous layer of hairy and non-hairy roots. 'Trichoblasts' are shaded, and root-hairs indicated by white circles. The broken lines delimit the arbitrary areas chosen for the determination of root-hair frequencies.  $\times 100$ .

by the two methods. It was, however, satisfactorily accounted for on the following lines. The potential hair-forming cells of the epidermis are much smaller than the others—commonly one-fifth in superficial area. In a drawing of a number of cells of the epidermis, therefore, the files of cells which make up the drawing will end in a complete hair-cell more often than they should. Since only complete cells were included in the area planimetered, this will artificially lower the observed value for the average cell area and will also increase the observed root-hair frequency. It is necessary, therefore, in making observations on roots with hair-forming cells very strongly differentiated (in point of size) to measure an arbitrary area and obtain the number of cells within it by adding together all the fractions of cells to the number of whole cells within the area. In this way a truer sample of the epidermis is obtained, and the root-hair frequency determined from it agrees much more closely with the value found by the section method. It must also give a truer value for the frequency of the epidermal cells, and different determinations agree more closely after the corrected

method has been used. The results are appended below. It will be seen that the hairy roots have consistently larger epidermal cells than the non-hairy, so that here, too, root-hair development appears to be correlated with better conditions for growth.

*Potamogeton densus.*

Hairy roots in stream.

Frequency of epidermal cells.		Frequency of hair-cells only.		Root-hair frequency by section method.
Uncorrected.	Corrected.	Uncorrected.	Corrected.	
per mm. <sup>2</sup>	per mm. <sup>2</sup>	per mm. <sup>2</sup>	per mm. <sup>2</sup>	per mm. <sup>2</sup>
263	257	112	105	96
312	284	151	119	
257	233	148	109	
Average values.				
277	258	137	111	

Hairless roots in stream.

357	330
298	294
282	296
Average values.	
312	307

Hairy roots grown in laboratory.

308	276	133	117	110
270	272	120	99	123
300	295	126	124	121
Average values.				
293	281	126	113	118

Hairless roots.

382	(As the hair-forming cells here were little differentiated in size no correction was applied.)
408	

In *Stratiotes aloides* there is also an early differentiation of the epidermal cells. Here, however, the potential hair-forming cells form a much smaller proportion of the epidermis, and in the young stages do not differ greatly in size from the non-hair-producing cells. *Stratiotes* is notable for the width of its root-hairs. They may be 100  $\mu$  in diameter and 5-10 mm. in length. As a result presumably of their great diameter they are quite rigid, and give the root a very stubby texture when they are short. As the hairs develop their bases enlarge considerably and displace the cells of the hypodermal layer, so that the adult hairs almost appear to have arisen from this layer. The erect branched papillae which arise on the inside of the base of the hair-cells, as described by Kroemer (7), were well developed in all but the youngest hairs. It is interesting to note also the presence of numerous large pits in the wall of the hair base (Fig. 5). These would, of course, facilitate the transference of substances from the hair, and do, indeed, suggest that the hairs of an aquatic plant such as this have some absorptive function, and are not merely a means of anchorage to the plant (see below).

*Stratiotes aloides.*

## Hairy roots.

## (a) proportion of functional hair-forming cells high

		Root-hair index.	
		%	
Cell frequency	75.3	Hair frequency	6.0
	47.7		6.7
	50.4/mm. <sup>2</sup>		5.3
Average	57.8/mm. <sup>2</sup>		6.0/mm. <sup>2</sup>

## (b) proportion of functional hair-forming cells low

Cell frequency	100	Hair frequency	5.75
	93		4.9
	112/mm. <sup>2</sup>		5.0
Average	105/mm. <sup>2</sup>		5.2

Hairless roots.	Cell frequency	161
		243
		210/mm. <sup>2</sup>

*Hydrocharis morsus ranae* is also remarkable for the size of its hairs, which may reach a length of 5-7 mm. A reduction in the intensity of hair formation is accompanied by a reduction in the size of the epidermal cells.

*Hydrocharis morsus ranae.*

## Hairy roots.

## Hairs well developed—3-4 mm. long.

		Root-hair index.	
		%	
Cell frequency	136/mm. <sup>2</sup>	Root-hair frequency	23.0/mm.
	160		27.5
	140		24.5

## Hairs poorly developed—very short.

	234		21.0
	314		19.0
	210		13.8

No hairless roots were observed or obtained in culture (except diseased specimens).

*Alisma plantago* is another species in which the hairs are developed only from specially differentiated cells. The latter do not appear to be formed in the hairless roots, though it is possible that under conditions which suppress hair formation these cells lose their special character and early take on a form indistinguishable from the non-hair-forming cells of the epidermis.

*Alisma plantago.*

## Hairy roots. Cell frequency

95	}
115	
131/mm. <sup>2</sup>	

Calculated from the non-hair-forming cells only, as the others are very small.

## Hairless roots.

## Cell frequency

137
150
163/mm. <sup>2</sup>

<sup>1</sup> The root-hair frequencies were approximately  $\frac{1}{2}$ - $\frac{1}{3}$  of these values, but were rather variable.

*Carex paludosa.*

This and other species of *Carex* are notable for the very regular development of the piliferous layer in which the hair-producing cells are formed in equal numbers and alternating with the non-hair-forming elements. Another

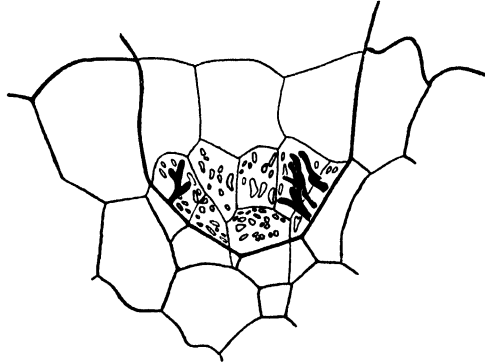


FIG. 5. *Stratiotes aloides*; Base of root-hair as seen in transverse section of the root.  $\times 250$ .

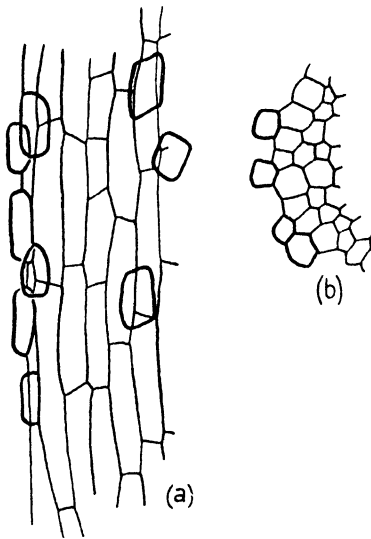


FIG. 6. *Carex paludosa*; Protruding trichoblasts, as seen in surface view and in transverse section.  $\times 215$ .

interesting feature sometimes appears, in that on some roots, particularly non-hairy ones, the hair-forming cells become extruded above the general level of the epidermis so as to appear as remnants of another layer of cells which has been almost completely removed. This process of extrusion may begin very early close behind the root-tip (see Fig. 6). As might be expected, it is accompanied by an increase in the length of the epidermal



cells. For this reason roots which show this extrusion phenomenon have been avoided in the numerical observations. The genus also appears to show some dimorphism in its root system. Thick, primary, adventitious roots are formed from the rhizome which do not form hairs. These are restricted to the finer ramifications of the root system. This is noticeable particularly in *Carex arenaria*.

*Carex paludosa*.

Hairy roots.	Cell frequency (per mm. <sup>2</sup> ).	Hair frequency (per mm. <sup>2</sup> ).	Root-hair index (%).
	1240	231	18
	1230	134	11
	1440	218	15
	1120	183	16

Hairless roots (not showing extrusion of hair-cells).

Cell frequency (per mm. <sup>2</sup> ).
2000
1990
2040

*Azolla*, while not normally producing root-hairs, does so under certain conditions. Root-hair development appears to be associated with increased aeration. It has been observed on plants which, as a result of a fall in the water-level, have been isolated in shallow pools with a comparatively large surface/volume ratio. Plants stranded on damp mud or grown on sand in a saturated atmosphere may also produce root-hairs. The hairs are developed from specially differentiated cells which are formed intermittently. The potential hair-forming cells in each group occur approximately at the same level so that the hairs which arise from them have a whorled formation. The production of root-hairs is accompanied by an increase in the dimensions of the epidermal cells as compared with those of roots without hairs.

*Azolla filiculoides*.

Hairy roots.			
Cell frequency	1250/mm. <sup>2</sup>	Hair frequency	250/mm. <sup>2</sup>
(average value)			Root-hair index 20 %.
Hairless roots.		Cell frequency.	
		3050	
		2270	
		2660	
		2760/mm. <sup>2</sup>	

*Callitriche stagnalis*, like *Elodea*, appears to develop its hairs only on roots which penetrate a substratum, though this was not closely investigated. Special hair-producing cells are not differentiated. Hair formation is here, too, accompanied by an increase in the size of the cells of the piliferous layer.

Hairy roots in mud.	Cell frequency.
	424/mm. <sup>2</sup>
	435/mm. <sup>2</sup>
Non-hairy roots.	583
	611
	624/mm. <sup>2</sup>

No attempt was made to estimate the frequency of the hairs.

In *Nasturtium officinale* there is a similar correlation between hair development and cell dimensions. Here the hairs are found principally on roots comparatively near the water surface, and are sparse or absent in roots at greater depths.

*Nasturtium officinale.*

Cell frequency	214	Hair frequency	99/mm. <sup>2</sup>	Root-hair index	46.0 %
	232/mm. <sup>2</sup>				
	258				
	385		49		12.8
	413				
	423				
	452				
	} hairs absent.				

*Myosotis palustris* (Hill) is interesting in that there is a very slight difference in the development of the epidermal cells of hairy and hairless roots. It is, of course, to be expected that different species will show different related proportional changes in root-hair development and epidermal cell size.

*Myosotis palustris.*

Hairy roots.	Cell frequency	283
		268
		264/mm. <sup>2</sup>
Hairless roots.		272
		278
		298/mm. <sup>2</sup>

*Scrophularia aquatica.* This is a particularly favourable species for investigation as the epidermal cells are comparatively large. The hairs are, therefore, less crowded. The positive correlation between root-hair development and epidermal cell development is well marked (Fig. 7).

*Scrophularia aquatica.*

Hairy roots.	Cell frequency.	Hair frequency.	Root-hair index.
			%
	213	114	53
	243	95	39
	225	88	39
	298/mm. <sup>2</sup>	99/mm. <sup>2</sup>	33
Hairless roots.	Cell frequency.		
	398		
	484		
	357		
	373/mm. <sup>2</sup>		

*Veronica becca-bunga.*

Root-hair formation in these species occurs principally on roots which are developed in water. Those which penetrate the (commonly) odoriferous mud are devoid of hairs. The hairy roots on which observations were made were not the most hairy observed. Many showed a better hair development, but it was so dense as to render observation impossible.

*Veronica becca-bunga.*

Hairy roots.	Cell frequency.	Root-hair index.
	427	} 12 % an approximate average value.
	537	
	473	
	485/mm. <sup>2</sup>	
Hairless roots.	Cell frequency.	
	656	
	642	
	727	
	715/mm. <sup>2</sup>	

*Ranunculus sceleratus.*

Hairy roots.	Cell frequency.	Hair frequency.	Root-hair index.
	273	109	%
	262	96	40
	314/mm. <sup>2</sup>	114/mm. <sup>2</sup>	37
			37
Hairless roots.	Cell frequency.		
	340	(A few hairs were present on this root.)	
	360		
	400/mm. <sup>2</sup>		

*Hydrocotyle vulgaris* is another favourable species for the investigation of root-hair development as it commonly occupies a considerable zone on the margins of ponds and thus extends over a fairly wide range of conditions.

*Hydrocotyle vulgaris.*

Hairy roots.	Cell frequency.	Hair development variable.
	350	} 20 % an approximate average value.
	274	
	317	
	296/mm. <sup>2</sup>	
Hairless roots.	Cell frequency.	
	442	
	404	
	498	
	443/mm. <sup>2</sup>	

In view of Schwarz's calculations of the same type, it was considered of interest, in those species for which the data is sufficient, to calculate the

increase in the superficial area of the root as a result of root-hair formation. If the average dimensions of the hairs are  $l$  mm. in length by  $b$  mm. in width and the hair frequency is  $n$  per mm.<sup>2</sup> the superficial area of the

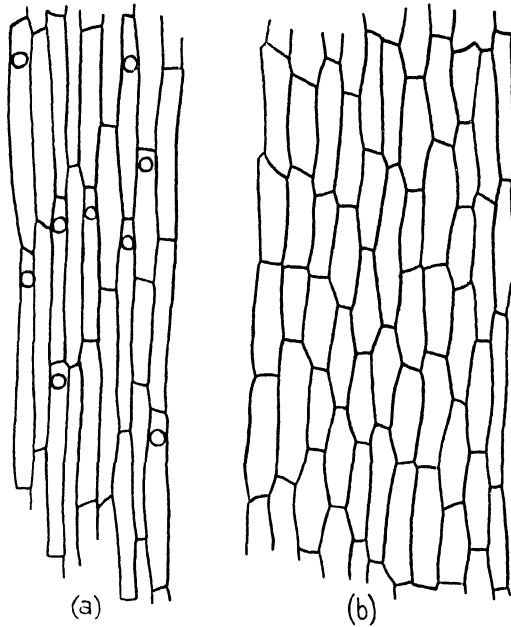


FIG. 7. *Scrophularia aquatica*; Piliferous layer of hairy and non-hairy roots.  $\times 115$ .

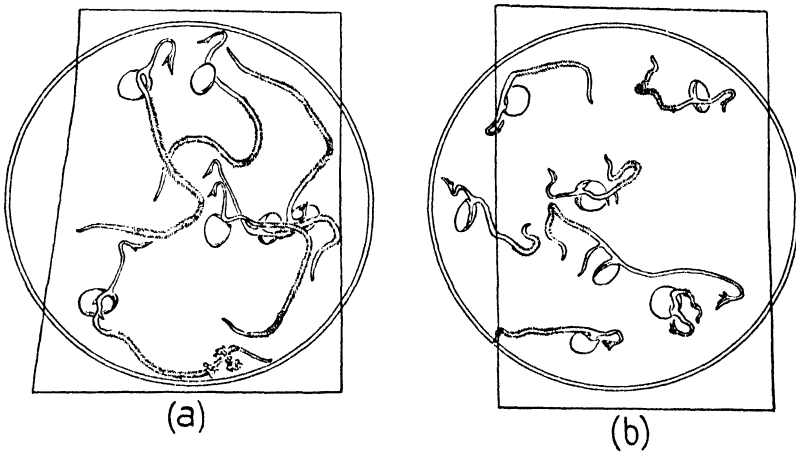


FIG. 8. *Lens esculenta*; Seedlings germinated on clean sand and on fresh damp earth under similar conditions of temperature and humidity.  $\times \frac{1}{2}$ .

hairs per mm.<sup>2</sup> of piliferous root is  $nlb\pi$  mm.<sup>2</sup> The proportional increase of surface is then  $(1 + nlb\pi) : 1$  for the hairy parts of the root.

For Lens seedlings in saturated air

$$n = 470, \quad l = 0.35, \quad b = 0.014.$$

Increase in area = 8.2 times.

Lens seedlings in saturated air + 10 %  
carbon dioxide.

$$n = 450, \quad l = 0.15, \quad b = 0.014.$$

Increase in area  $\times 4$ .

*Elodea*.

$$n = 50, \quad l = 1.5, \quad b = 0.015.$$

Increase in area  $\times 4.5$ .

*Potamogeton densus*.

$$n = 100, \quad l = 0.8, \quad b = 0.02.$$

Increase in area  $\times 6$ .

*Stratiotes aloides*.

$$n = 6, \quad l = 5, \quad b = 0.09.$$

Increase in area  $\times 9.5$ .

*Hydrocharis morsus ranae*.

$$n = 25, \quad l = 3, \quad b = 0.05.$$

Increase in area  $\times 14.8$ .

*Azolla filiculoides*.

$$n = 250, \quad l = 0.25, \quad b = 0.01.$$

Increase in area  $\times 3$ .

*Carex paludosa*.

$$n = 190, \quad l = 0.7, \quad b = 0.011.$$

Increase in area  $\times 5.6$ .

*Scrophularia aquatica*.

$$n = 100, \quad l = 0.6, \quad b = 0.014.$$

Increase in area  $\times 3.6$ .

*Ranunculus sceleratus*.

$$n = 100, \quad l = 0.4, \quad b = 0.012.$$

Increase in area  $\times 2.5$ .

*Nasturtium officinale*.

$$n = 90, \quad l = 0.5, \quad b = 0.011.$$

Increase in area  $\times 2.5$ .

*Callitriche stagnalis*.

$$n = 380, \quad l = 0.5, \quad b = 0.009.$$

Increase in area  $\times 6.4$ .

*Alisma plantago*.

$$n = 40, \quad l = 0.3, \quad b = 0.015.$$

Increase in area  $\times 1.6$ .

*Hydrocotyle vulgaris*.

$$n = 70, \quad l = 0.5, \quad b = 0.015.$$

Increase in area  $\times 2.6$ .

*Veronica becca-bunga*.

$$n = 60, \quad l = 0.7, \quad b = 0.011.$$

Increase in area  $\times 2.4$ .

As Farr (5) points out, calculations of the type given above must, in any case, be taken with caution, as an increase in the superficial area of the root does not necessarily carry with it a proportionate increase in the power of absorption. Substances which tend to pass into the hair, whether they be water or salts, must all pass across the base of the hair into the root, and ultimately, of course, across the endodermis. Thus there will be these two more or less independent limiting factors—the area of the hair base acting on absorption by the hair, and the endodermis exerting a limiting influence on the rate of absorption generally. The limiting effect of the hair base will be relatively greater for long than for short hairs, and we see that long-haired species have also wider hairs than the others, though this condition is probably brought about as much by demands of mechanical strength as by its effect on the process of absorption. Any increase in absorptive area can only be of value when these factors are not limiting, i.e. under conditions of relative deficiency of the substances being absorbed. In the face of this conclusion one is struck by the characteristically great hair production, with quite high increase in absorptive surface, e.g. *Hydrocharis*  $\times 14.8$ ; of many aquatics, such as *Elodea*, *Stratiotes*, *Potamogeton*, *Callitriche*, and *Trianea* (described by Schwarz).

This point was appreciated by Schwarz, who suggested, as a teleological explanation of the facts, that in aquatics the chief function of the hairs is that of anchorage. The apparent paradox is also interesting in connexion with the theory put forward by Scott and Priestley (16), that the absorption of water and salts are two separate processes. According to these authors, a root in a water-logged soil is capable of absorbing water independently of the action of the protoplasts of the piliferous layer or, indeed, of the cortex. The cell-walls being permeable, water will be able to pass into the root along these, as distinct from passing through the cells, and will ultimately reach the endodermis, where its further progress by diffusion in the cell-walls will be arrested by the suberized radial walls of this layer. The endodermis, as a whole, then, functions as a semi-permeable membrane across which the water enters the stele.

This, of course, accounts only for the absorption of water. The absorption of mineral salts presumably takes place to a greater or lesser extent by all cells which come into contact with the soil solution. If this theory be applied to the root systems of aquatic plants it is at once apparent that root-hairs would confer no benefit on the root in so far as absorption of water is concerned, as other internal factors would be limiting. At the same time the concentration of salts in the aqueous medium is very low, and it seems probable that the production of root-hairs is important in the absorption of these salts, their concentration being sufficiently low, that the internal factors, although limiting in the absorption of water, are not so in respect of the solutes. In this connexion no reference has been made to the view put forward by Coupin (2), (3), (4), that the root-tip is the principal absorptive region of the root, as this has already been discussed by Priestley and Tupper-Carey (12). Neither need the theory of absorption of solid particles by root-hairs, as described by Jamieson (6), be seriously considered.

With the foregoing considerations in mind any calculations relating to the absorptive surface of the root appear to be of uncertain significance, especially in the absence of any exact data as to the relationships between the external and internal factors controlling the absorptive process. They do, however, convey some impression of the capacity of the plant in the direction of increasing its surface of absorption, and it seems reasonable to assume that under some conditions this would be limiting in value. Any increase in the absorptive surface would then be correspondingly beneficial to the plant.

The correlation between root-hair formation and an increase in the dimensions of the epidermal cells would appear to have a double significance. In the first place, the root-hair cannot be regarded as a lateral outgrowth stimulated by retardation of the tip of the root (Mer), nor as

a resultant of the difference between the elongating capacity of the epidermal cell and the retarding influence of the cortex (Snow). It is an enlargement of the cell which takes place when the conditions for enlargement are suitable, and under these conditions the cell is able to enlarge in a longitudinal direction also. It is an outgrowth of the cell when conditions for growth are most favourable. This was the conclusion reached by Schwarz. This applies to a stabilized condition of hair formation. At the initiation of a period of hair formation there may be a temporary derangement of the development of the cells of the piliferous layer.

Secondly, the frequency of the hairs (expressed as the number per unit area) cannot be regarded as a satisfactory criterion of root-hair development, for if root-hair production is associated with an increase in the size of the piliferous cells the maximum root-hair frequency is thereby lowered. Observations on *Lens* seedlings, as well as some of the figures given above (*see* *Stratiotes* and *Hydrocharis*), show that changes in the environment which markedly affect the growth rate and the length of the hairs may not be accompanied by any significant change in the frequency of the hairs. A better indication of the intensity of the root-hair development would appear to be not the frequency of the hairs alone, but this frequency expressed in relation to the frequency of the cells of the piliferous layer; in other words, the proportion of the epidermal cells which produce hairs. This is, of course, analogous to the concept of the stomatal index (14) as applied to the consideration of the development of stomata in leaves, and may for convenience be termed the root-hair index.

Then

$$\text{root-hair index} = \frac{\text{frequency of root-hair-forming cells}}{\text{frequency of all epidermal cells}} \times 100.$$

If root-hair production is to be correlated with any other of the plant's activity the root-hair index would appear to supply the most satisfactory unit of measurement. Frequency alone cannot be regarded as giving a true indication because it is the result of two separate factors. The frequency of the epidermal cells is important in that it sets a maximum to the hair frequency which is theoretically possible, and the proportion of the epidermal cells functioning in hair production may reduce this maximum in almost any proportion. If these two factors operate in opposite directions then they may cancel one another's effects as far as the root-hair frequency is concerned.

The comparison of the root-hair index with the stomatal index must not be driven too far. In the course of its formation and development a root may be subject to a much greater variety of environmental conditions than is a leaf. The leaf rudiments are laid down and begin their development in the bud, under conditions which are more or less independent of those obtaining outside. In contrast to this, the embryonic region of the

root is in close contact with external conditions, and as it develops it is being pushed through the soil and is liable to be exposed to a considerable range of conditioning factors. As might be expected under these conditions, the root-hair is a relatively unstable and opportunist structure. It develops some little way behind the root-tip, and hence will be affected by different conditions during its inception than those affecting the epidermis generally. The root-hair index thus shows a much greater variability in a species than does the stomatal index. Nevertheless, it is in many plants potentially constant.

Schwarz (15), Leavitt (8), van Tieghem (18), and others have noted that in many Monocotyledons and Pteridophytes the epidermal cells of the root are differentiated early in the ontogeny of the root—(see Elodea, Potamogeton, Azolla, Stratiotes, Carex, &c., above)—into piliferous and non-piliferous elements. The former, which are capable of producing hairs later are distinguished in their early stages commonly by being thicker walled or possessing a larger nucleus and denser cytoplasm than the others. They are also usually smaller, this difference being entirely one of length. This differentiation is apparent a short distance behind the root-tip, and long before the hairs are actually developed. The interesting feature of this development is that under widely different conditions of growth, and accompanied by considerable differences in the size of the epidermal cells, the proportion of root-hair-forming cells (called trichoblasts by Leavitt) laid down at the root-apex is remarkably constant for a species. That is, in these plants the root-hair index is a potentially constant quantity. But in actual fact this potential constancy is destroyed by the variability in the root-hair-forming activity of the trichoblasts. All of them may function, or none may do so.

The variability in hair formation shown by the epidermis is probably attributable to the close connexion which exists between the root-hair and the surrounding medium. A glabrous epidermis has a relatively small proportion of its surface exposed to the outside. As soon as hairs are formed this proportion is rapidly increased, so that its sensitivity to external conditions would be expected to show a corresponding rise. If these conditions become unfavourable a reduction in the length of the hair would afford the most rapid adjustment to the new environment. This probably accounts for the much greater variability in the proportion of the epidermal cells which produce hairs, and in the length of the hairs, than in the size of the epidermal cell itself.

In their effect on the root-hair frequency these factors act in opposite senses. A reduction in the area of the epidermal cell will tend to increase the root-hair frequency, while a reduction in the root-hair index, which appears to be concurrent with the other, will tend to decrease it. For this reason the root-hair frequency may show considerable variation, and a



change in conditions of growth may raise or lower the root-hair frequency according as that change produces a greater or less (proportional) change in the average cell area than in the root-hair index. It seems possible that a consideration of this type might explain the opposite conclusions of different workers that the root-hair production is an accompaniment of increased or decreased growth rate.

The correlation between kinking of the root and the root-hair development illustrates this. Mer regarded kinking of the root (of *Lens*) as due to growth retardation; it was accompanied by good root-hair development. Schwarz also correlated kinking of the root with good hair formation, but regarded kinking as evidence of maximum growth energy.

The kinked condition can readily be obtained by growing seedlings of *Lens* on the surface of damp soil in an approximately saturated atmosphere. The radicles run about the surface of the soil and show sudden bends, laterally or even in an upward direction. This kinking does not occur (or at least to a very much smaller extent) in seedlings grown on clean damp sand or filter-paper under otherwise similar conditions.

Two small earthenware dishes were prepared, one of fresh damp earth and the other of damp sand. A number of soaked *Lens* seeds were placed on the surface of each and the dishes were covered with glass over about three-quarters of their top. They were then placed in a larger, almost completely covered glass trough containing water. They were thus under similar conditions of humidity. After a week the radicles had reached average lengths of approximately 8 cm. and 4 cm. on the sand and soil respectively. The difference between the degree of kinking of the radicles in the two dishes was well marked. The seedlings on the soil were very considerably more kinked than the others, as can be seen from the drawings. A repetition of the experiment gave similar results (Fig. 8).

The seedlings of each batch were examined for root-hair development, areas being chosen for this purpose at approximately the middle of the root-hair zone, and in the kinked radicles, away from sharp bends. In a plant such as *Lens*, where the root-hair development may be very dense, observations of the frequency of the hairs and of the epidermal cells would present some difficulty, but it was found that with practice the epidermis could be stripped off with a minimum of cortical tissue (1–2 cell layers) and placed on a slide with its external surface downwards. The epidermis could then be examined from its inner side, and those cells which produce hairs were readily distinguishable, as the base of the hair appears as a circle within the cell outline. Camera lucida drawings made in this way formed a basis for calculations of the actual area, and hence frequency of the epidermal cells. From the ratio of hair-producing to total number of cells the root-hair frequency can be arrived at. The figures indicate that the kinked seedlings, whilst showing a root-hair frequency slightly higher than

that of the unkinked, have a significantly lower root-hair index and smaller epidermal cells, which, in conjunction with the obviously smaller growth, supports Schwarz's contention that root-hair development is an accompaniment of good growth conditions. He appears to be wrong, however, in associating kinking with a greater growth rate.

*Lens Seedlings Grown on Soil and Sand. Comparison of Root-hair Development on 'Kinked' and 'Unkinked' Seedlings.*

'Kinked'.			'Unkinked'.		
Frequency of cells.	Frequency of hairs.	Root-hair index.	Frequency of cells.	Frequency of hairs.	Root-hair index.
per mm. <sup>2</sup>	per mm. <sup>2</sup>	%	per mm. <sup>2</sup>	per mm. <sup>2</sup>	%
712	504	70.6	708	584	82.5
732	500	68.2	495	470	95.0
757	497	65.8	620	520	83.8
830	554	66.7	593	504	84.8
806	538	66.7	546	502	92.0
750	544	72.5	528	386	73.2
			558	438	78.6
			650	503	77.4 <sup>1</sup>
Average values.					
764	523	68.4	587	488	83.4

After penetration of the soil substrate.

Frequency of cells.	Frequency of hairs.	Root-hair index.
per mm. <sup>2</sup>	per mm. <sup>2</sup>	%
565	—	—
597	—	—
718	—	—
945	465	44

The 'kinking' is presumably due to substances given off by the soil (CO<sub>2</sub>?), or the greater surface exposed by the soil may have contributed to render the atmosphere in contact with it more humid. However, 'kinking' could not be induced to occur in seedlings grown on sand or blotting-paper in saturated atmosphere. Both over the soil and sand the water content of the atmosphere must have been approaching saturation at least, as condensation was usually present on the glass slips.

An examination of some parts of the roots which had penetrated the substrate showed that in the sand the root-hair index and dimensions of the epidermal cells were little affected.

In the soil the hairs were absent on the regions near the surface and the epidermal cells were somewhat larger than those of the exposed parts. An explanation of this is advanced below in relation to experiments on transferring *Lens* seedlings from air to air containing 10 per cent. CO<sub>2</sub>. More distal regions of these roots showed a reduced epidermal cell size and a rather sparse hair development.

<sup>1</sup> On soil, but not kinked.

In connexion with Mer's observations as to the relative root-hair development on the concave and convex sides of a bend in a root, it is interesting to apply the root-hair index. The epidermis and root-hair development were observed here, as in the previous experiment, by stripping the epidermis and examining it from the inner surface. As would be expected, the outer (convex) side showed larger (chiefly longer) epidermal cells than the concave side. It also showed a higher proportion of hair-producing cells. A calculation of the frequencies on the two sides shows little difference between these quantities. Except by some such strip method as that described above, it would appear not to be possible to estimate the root-hair frequency on the inner and outer sides of a bend in a root. The method of cutting sections of known thickness and counting the hairs on each while being quite satisfactory on a straight piece of root cannot be applied on a bend. It is possible to split the root longitudinally in a direction perpendicular to the plane of the bend, and then to straighten out each half and section them independently. This, however, tends to compress the convex and extend the concave surfaces, thus artificially raising and lowering the observed value of the root-hair frequency respectively on the two sides. Mer does not state how he observed the better root-hair development on the convex side of the bend. Had he used a method such as this it would account for his results.

*Lens esculenta. Comparison of Root-hair Development on Concave and Convex Sides of a Sharply Curved Root.*

Concave side.			Convex side.		
Frequency of epidermal cells.	Frequency of hairs.	Root-hair index.	Frequency of epidermal cells.	Frequency of hairs.	Root-hair index.
per mm. <sup>2</sup>	per mm. <sup>2</sup>	%	per mm. <sup>2</sup>	per mm. <sup>2</sup>	%
1140	660	58	665	485	73
828	512	62	580	448	77
815	375	46	524	352	67
813	416	51	580	403	69
762	350	46	622	447	72
1450	865	60	925	707	80

It will have been noted that the observations on the connexion between root-hair development and the size of the epidermal cells made on plants growing under natural conditions were all done on aquatic or sub-aquatic species. The roots of such plants are developed in conditions which are relatively constant over considerable lengths of the root, and it is thus comparatively easy to find representative regions for detailed examination. Under more terrestrial conditions the range of variation in environment is much greater, as, for instance, in relation to temperature, humidity, and aeration. The development of root-hairs may then become correspondingly irregular. There will be corresponding irregularities in the development of the epidermal cells, though these are often in the opposite sense to

those instances detailed above. In this connexion it is important to note that as the root-hair is developed some distance behind the root-tip it will take an appreciable time before the epidermis and root-hairs can come into equilibrium under the new environment. This effect is largely eliminated in aquatic plants, which have the added advantage that soil is more easily removed from the roots without damage to the hairs. This consideration is of considerable practical importance.

The effects of changes in growth conditions on the development of the piliferous layer was investigated in Lens. Seedlings were used as they provide suitable material for examination, and the depressive agent employed for reducing the development of the root-hairs was carbon dioxide. An atmosphere containing approximately 10 per cent. by volume of carbon dioxide was found to provide a suitable environment for inducing reduced root-hair formation.

The seeds used for these experiments were soaked in cold water for 20-24 hours before use. They were germinated in glass-stoppered jars of approximately 600 c.c. capacity. In each jar a slip of glass was wedged into the underside of the stopper with pieces of cork so that it extended almost to the bottom of the jar. This cork was sealed over with 'vaspar' so that its interstices would not become breeding-grounds for bacteria or fungi. The glass slips were wrapped in filter-paper, held in place by elastic bands, and the seeds were attached by similar means, usually six on each slip. After germination had begun two seedlings were removed so as to leave four of as uniform development as possible. The bottles were calibrated on strips of sticking-plaster adhering to the outer surface—this remains in position unaffected by water. For filling with gas the bottles were filled first with water and inverted in a trough. Matters were so arranged that the small amount of water necessarily remaining in the neck of the bottle after the gas had been admitted was just sufficient on re-inversion to bathe the tip of the glass slip and filter-paper to which the seeds were attached. The carbon dioxide used for the experiments was prepared in a Kipp's apparatus from marble chips and half-strength commercial hydrochloric acid, and passed through a wash-bottle before use. It also received some subsequent washing as the water in the trough was 4-5 inches deep. No attempt was made to obtain it specially pure, as the object of the experiment was not so much to study the effect of carbon dioxide as such on the root, but to observe the phenomena associated with growth retardation. Carbon dioxide prepared in this way was found to furnish a convenient means of attaining this end. After the correct amount of carbon dioxide had been passed into the jar the necessary air was pumped in from a bicycle inflator fitted with a non-return valve. The gas mixtures so obtained were replaced daily.

The root-hair development was investigated by stripping the epidermis

and examining it from the inner surface. The results obtained from strips taken from seedlings grown in air and in air containing 10 per cent.  $\text{CO}_2$  are given below. They show a well-marked association of reduced root-hair development with reduced size (increased frequency) of the epidermal cells (see Fig. 9).

*Lens.*

Roots in saturated air.			Roots in saturated air + 10 % $\text{CO}_2$ .		
Frequency of epidermal cells.	Frequency of hairs.	Root-hair index.	Frequency of epidermal cells.	Frequency of hairs.	Root-hair index.
per mm. <sup>2</sup>	per mm. <sup>2</sup>	%	per mm. <sup>2</sup>	per mm. <sup>2</sup>	%
538	415	77	955	487	51
583	458	78	630	390	62
554	465	84	782	485	62
576	427	74	616	400	65
493	375	76	815	489	60
598	444	74	808	508	63
768	632	82	874	473	54
568	510	89	515	312	61
656	558	85	542	363	67
770	612	79	950	485	51
506	377	74	750	472	63
630	500	79	774	464	60
473	396	84	756	522	69
584	404	69			
Average values.					
592	468	79	751	451	60

It should perhaps be mentioned that these figures are somewhat misleading in their magnitude. The area conveniently observable at one time under the microscope embraces thirty-five to sixty cells only. The frequencies have been calculated from observations of this order and are expressed in terms of the square millimetre merely as a convenient unit.

It will be noted that while the presence of 10 per cent. of carbon dioxide reduces the root-hair index from 79 to 60 the compensating effect of the accompanying reduction in the size of the epidermal cells keeps the root-hair frequency at a value close to that obtaining in air. It is scarcely surprising, therefore, that estimates of the development of root-hairs based on determinations of the root-hair frequency lead to discordant conclusions.

Lens seedlings which have been started in saturated air and transferred to air containing 10 per cent.  $\text{CO}_2$  show an anomalous behaviour comparable with that which has been noted elsewhere in this and other species.

The radicles of Lens seedlings grown in air usually develop their hairs some 8–15 mm. behind the root-tip. At the time of the transfer to the 10 per cent. carbon dioxide the position of the root-tip and of the lower extremity of the root-hair region were marked on the filter-paper in front of which the seedlings were growing. After three to four days' growth in the 10 per cent. carbon dioxide the region between the marks which was hairless before (because it was immature) had remained almost so, hairs

being formed only sporadically in this region. The epidermal cells, however, reached a size slightly exceeding that of those produced normally in saturated air. The region of the root below this more or less glabrous

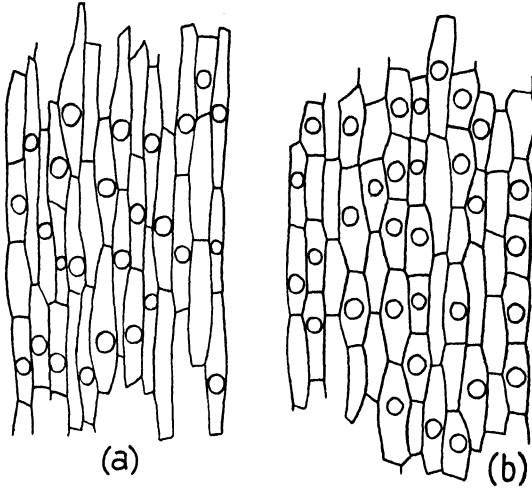


FIG. 9. *Lens esculenta*; Piliferous layer of seedlings grown (a) in saturated air, and (b) in saturated air with 10%  $\text{CO}_2$  (nominal), showing root-hair formation.  $\times 145$ .

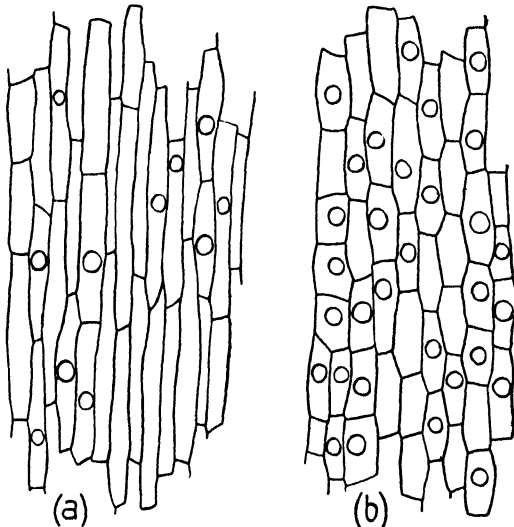


FIG. 10. *Lens esculenta*; Piliferous layer of seedling transferred from saturated air to saturated air with 10%  $\text{CO}_2$ ; (a) intermediate, almost glabrous zone, (b) second hairy zone. The hairs of the first hairy zone, developed in air, had shrivelled, and comparison must be made for this region with Fig. 9a.  $\times 145$ .

part produced a new crop of hairs, but its epidermal cells remained short like those of roots growing throughout in 10 per cent. carbon dioxide.

The new hairs were developed to an intensity approximating to that of seedlings normally developed in 10 per cent. carbon dioxide. The initiation of the new hairy zone occurs at a point roughly corresponding to the position of the root-tip at the time of the transfer, though it may take place a little earlier or later. At the end of the experiment, therefore, the root shows a hairy zone, followed by a more or less glabrous one in which an increase in epidermal cell length is apparent, followed again by a second hairy zone where a reduction in the dimensions of the epidermal cells has taken place. The frequencies of the hairs in the two zones are not greatly different, so that the illusion is created that root-hair development is accompanied by reduced growth rate. It is only when the root-hair indices of the two zones are determined that it becomes apparent that a reduction in the intensity of root-hair development has taken place. It was found at the end of the experiment that the root-hairs developed prior to the transfer had shrivelled in the new environment. There is, however, no reason to suppose that their development was abnormal as compared with seedlings grown entirely in saturated air, so that the figures for these seedlings were used for comparison (Fig. 10).

*Lens Seedlings Transferred from Air to Air Containing 10 per cent. CO<sub>2</sub>.*

Intermediate zone. (usually almost glabrous).		Second hairy zone.		
Frequency of cells. per mm. <sup>2</sup>	Frequency of hairs.	Frequency of epidermal cells. per mm. <sup>2</sup>	Frequency of hairs. per mm. <sup>2</sup>	Root-hair index. %
390	sparse	825	520	63
556	"	647	390	60
—	copious	570	386	68
470	sparse	1050	638	61
385	266/mm. <sup>2</sup>	925	570	62
410	sparse	{ 960	590	62
		{ 610	408	67
605	"	692	440	63
424	"	684	445	67
295	"	{ 937	535	57
		{ 835	490	59
		{ 840	552	67
		Average values.		
442		768	484	64

Corresponding averages for roots developed throughout in air and in air containing 10 per cent. carbon dioxide.

539/mm.<sup>2</sup> (468/mm.<sup>2</sup> 79%) 751/mm.<sup>2</sup> 451/mm.<sup>2</sup> 60%.

The differential effect of the change of environment on the epidermal cells and the hairs would appear to be explicable on the following lines: since the hairs are formed some distance behind the root-tip, at any given

point on the root, the hair has undergone its development at a later stage than the basal region of the epidermal cell. Hence the change of environment might well affect the hair to a greater extent than the epidermis, as it is acting at an earlier stage in its life. Thus the glabrous region of the root which was immature at the time of the transfer has remained hairless, or at least suffered a great reduction in hair development, because the carbon dioxide was acting at the inception of root-hair development; its action on the epidermal cell is relatively later, and hence might well be less marked. Why the epidermal cells in this region should increase in dimensions is not so clear, particularly if the effect of the carbon dioxide on the cell is regarded as a depressive one. It may be that the cessation of the production of hairs creates in the epidermis a temporary superfluity of food materials, the effect of which in causing enlargement of the cells outweighs the depressive action of the carbon dioxide. This interpretation is in accordance with the development of hairs in the younger region. It is at least reasonable to suppose that the carbon dioxide would not exert so powerful a depressive action on development in the direction of cell enlargement as it would on hair production, as the epidermis will not suffer by the exposure of so great a proportion of its superficial area as would be developed if root-hairs were formed. At the distal end of the glabrous region the carbon dioxide has acted almost since the formation of the epidermis—its effect is, therefore, more pronounced, and the cells are correspondingly shortened. Lower down the epidermis has been laid down and developed entirely in 10 per cent. carbon dioxide, and the development thus takes the form characteristic of this environment. Epidermal cell growth and hair production have here been more or less equally depressed in the young stage, and in the absence of the differential effect there is no diversion of food material to the one aspect of cell growth at the expense of the other.

There seems no reason to doubt that this sequence of changes in the piliferous layer observed in this experiment is similar to changes which occur under natural conditions, both in *Lens* and in other species. It would appear, however, unlikely that the alternation of hairy and non-hairy zones, with the accompanying changes in the development of the cells of the piliferous layer, is always due to a deterioration of the conditions of root-hair development. This would mean that in a root which shows a number of successive hairy zones there is a progressive deterioration of the root-hair development. An example of this type of root-hair formation is furnished by *Eupatorium cannabinum*, which appears regularly to produce its hairs in zones alternating with non-hairy regions, and not merely at the base of the root, as stated by Whitaker (19), and the intensity of hair development in successive zones may not show great variation. This feature is noticeable both in plants growing under natural conditions and also in seedlings germinated on damp sand. An examination of the



piliferous layer of these zones shows that the cells of the glabrous zones are regularly slightly larger than those of the non-hairy, a condition comparable with that of the *Lens* seedlings transferred from air to air with 10 per cent. carbon dioxide. Any change in conditions may, therefore, bring about a discontinuity in root-hair formation. Attempts to induce *Eupatorium* to produce continuous zones of hairs so that these might be compared with hairless roots were unsuccessful.

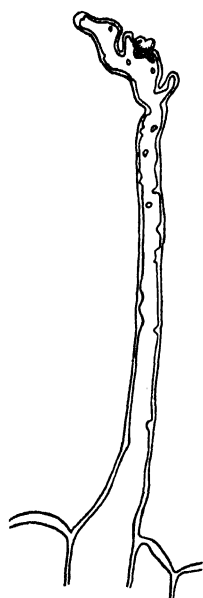


FIG. 11. *Eupatorium cannabinum*; Thick-walled root-hair.  $\times 250$ .

One may mention another feature in which the root-hairs of *Eupatorium* are of interest, a feature which is shared also by *E. cordatum* and *E. purpureo-album*. The root-hairs of these plants become thick walled at an early stage, and in this condition, in common with the outermost 2-3 layers of the root, give a faint but definite reaction with phloroglucin and hydrochloric acid. They do not swell up and turn blue in iodine and sulphuric acid, but merely take on a faint greenish-blue coloration without losing their shape, so that it is evident that the wall of the hair is not entirely of a cellulose nature. The hairs may become branched, and the inner surface of the walls bear papillae which approach in proportions those of the tuberculate rhizoids of the Marchantiaceae (Fig. 11). The hairs are very persistent and are commonly to be found on parts of the roots in which secondary thickening is quite

marked, a feature which conflicts with the theory put forward by Whitaker (19). The primary structure of the cortex is retained for some time, though numerous cell divisions in the radial direction occur in this region as a result of the increasing girth of the stele.

That the retention of the hairs to so late a stage is not merely a purely mechanical consequence of their robust nature is shown by the fact that most of them are still alive at this stage and capable of being plasmolysed.

In view of the nature of root-hair development in *Eupatorium* the experiment was tried of transferring *Lens* seedlings from 10 per cent. carbon dioxide to air to ascertain if this would also bring about the formation of a transitional hairless region before the new hairs are formed.

The method adopted was to germinate the seedlings in 10 per cent.  $\text{CO}_2$  for 5-6 days, when their radicles had reached a length of 15-20 mm., and root-hairs had appeared in an intensity usual for the environment. They were then allowed to continue for a few days in air until sufficient new growth had been made for the root-hair development under the new conditions to be estimated.

Lens seedlings so treated were affected to an unlooked-for and very marked degree by the change.

In the majority of seedlings which undergo this transfer the primary root turns brown at the tip and growth there ceases, to be carried on by

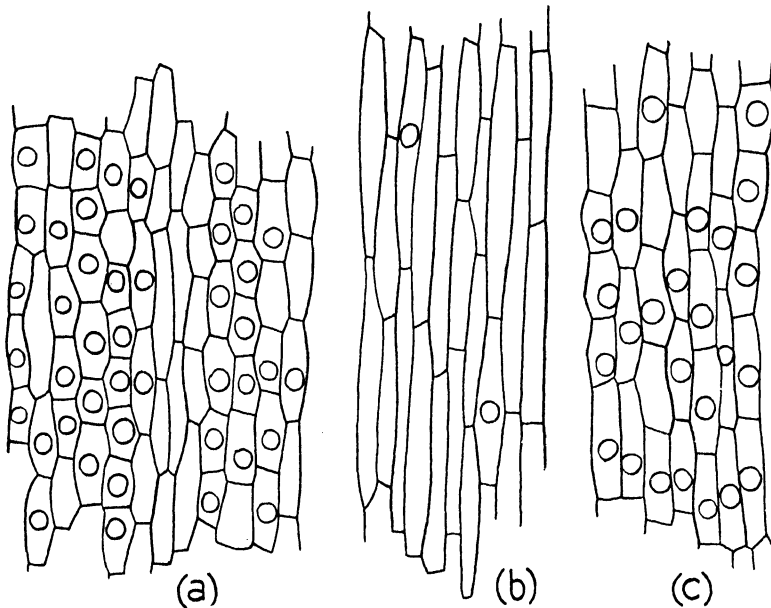


FIG. 12. *Lens esculenta*; Piliferous layer of seedling transferred from saturated air with 10 %  $\text{CO}_2$  (nominal) to saturated air; (a) first hairy zone developed in 10 %  $\text{CO}_2$ , (b) intermediate zone, almost devoid of hairs, (c) second hairy zone developed in air.  $\times 145$ .

lateral roots, which are unaffected, probably because in general they arise subsequent to the change in the environment.

The shrivelling of the tip of the primary root occurs in approximately 70 per cent. of the seeds germinated, and also, as a rule, within a comparatively short time of the transfer. Of 48 radicles which shrivelled in this way, the shrivelling occurred on an average after the root had elongated 9 mm. subsequent to the transfer. 65 per cent. of the shrivelled roots made no more than 1 cm. growth in this way.

In those which continued to grow subsequent growth consisted of a glabrous or sparsely haired zone of varying length followed in some instances by a well-defined new hairy zone which approximated in its intensity of hair formation to that characteristic of *Lens* seedlings grown in air. There seems no reason to suppose that the others would not eventually have produced this new zone of hairs, but in a great many cases the intermediate glabrous zone was so extensive that the radicle was closely approaching the surface of the water in the bottom of the jar before the new zone of hairs was initiated, and hence in these instances the experiment could not be

completed. The complete cycle of change in root-hair development was, however, fully observed in 11 seedlings out of a total of 75, of which, as was stated above, 48 suffered shrivelling of the primary root. Actually, a slightly greater number of seedlings did complete the cycle, but the observations were spoiled owing to bad stripping of the epidermis.

The cycle of changes is as follows: the region of the root, which at the time of the change was hairless (being the immature region immediately behind the root-tip), becomes hairy, and the hairs in the region often reach a length of the same order as that normal for hairs developed in air—they are certainly longer than those developed in 10 per cent. carbon dioxide.

However, the root-hair index in this region does not appear to increase. There is, in this way, a tuft of long hairs produced in the last 2–3 mm. before the change point. The immediate subsequent development is as a rule hairless, though hairs are sometimes formed in low intensity. This is the intermediate glabrous or semi-glabrous zone. Development up to this stage can be observed in all roots, though in those which shrivel this intermediate zone is quite devoid of hairs. In the other roots the intermediate may extend to a considerable and variable length until the new hairy zone supervenes, if it does so at all, within the compass of the experiment.

Parallel with these experiments control seedlings were, of course, grown for corresponding periods, being kept in 10 per cent.  $\text{CO}_2$  the whole time. Notes were made as to the length of these seedlings in the periods corresponding to the pre- and post-transfer growth of the others. Observations were also made on the root-hair development. Detailed calculations were carried out in a proportion of instances to show that there was no significant change in the root-hair index over the whole hairy region of the root. In the remainder it was considered sufficient to observe that the hair development was reasonably uniform and of the type usually developed in 10 per cent. carbon dioxide. This could be determined by inspection.

The numerical results of this experiment are given in the table on p. 151.

As in the reverse transfer from air to 10 per cent. carbon dioxide, the results show the production of a glabrous zone between the two hairy zones which is characterized by possessing larger epidermal cells than either of the hairy zones (Fig. 12). It will be noted that the frequencies of the cells of the piliferous layer in all three zones are consistently higher than the frequencies of corresponding zones in previous experiments. Thus the first zone developed in 10 per cent. carbon dioxide had cell frequency  $855/\text{mm}^2$ , root-hair index 61.2 per cent.; intermediate zone, cell frequencies  $503/\text{mm}^2$ , and the second hairy zone in air, cell frequency  $694/\text{mm}^2$ , root-hair index 74.3 per cent. Corresponding frequencies for previous experiments gave in

*Lens transferred from 10 per cent. CO<sub>2</sub> → air.*

No.	In 10% CO <sub>2</sub> .		Root-hair index.	Intermediate zone.		In air.	
	Frequency of cells.	Frequency of hairs.		Frequency of cells.	Frequency of cells.	Frequency of hairs.	Root-hair index.
	per mm. <sup>2</sup>	per mm. <sup>2</sup>	%	per mm. <sup>2</sup>	per mm. <sup>2</sup>	per mm. <sup>2</sup>	%
3 <i>d</i>	690	435	63	484	Some indication of new hair-zone appearing.		
3 <i>h</i>	675	450	66.7	355	830	615	74.3
4 <i>b</i>	967	598	62	523	615	433	70.4
5 <i>b</i>	726	392	54	916	662	448	68
6 <i>a</i>	697	418	60	484	674	493	73
10 <i>g</i>	832	560	66	—	875	650	74.3
11 <i>b</i>	751	494	65.6	383	854	610	71
11 <i>c</i>	1290	720	55.5	405	626	498	79.5
11 <i>f</i>	1200	700	58.2	572	516	392	76
14 <i>a</i>	722	423	58.5	488	780	623	80
15 <i>f</i>	855	547	64	416	645	494	76.5
					638	570	80.0
	Average values.						
	855	522	61.2	503	694	514	74.3

*Controls kept in 10 per cent. CO<sub>2</sub>.*

No.	Frequency of cells.		Root-hair index.	Frequency of cells.		Root-hair index.
	per mm. <sup>2</sup>	per mm. <sup>2</sup>		per mm. <sup>2</sup>	per mm. <sup>2</sup>	
4 <i>b</i>	971	631	65	936	583	62.2
6 <i>c</i>	995	560	56.5	1045	640	61.3
10 <i>b</i>	898	570	63.5	905	525	58.3
11 <i>b</i>	802	505	63	960	547	57
12 <i>b</i>	930	530	57	870	487	56
12 <i>c</i>	1040	640	61.7	1210	563	59
14 <i>c</i>	1080	604	56	972	715	58
	Average values.					
	959	566	60.1	985	580	58.8

air, cell frequency 592/mm.<sup>2</sup>, root-hair index 79 per cent.; intermediate zone, cell frequency 442/mm.<sup>2</sup>; in 10 per cent. carbon dioxide cell frequency 768/mm.<sup>2</sup>, root-hair index 64 per cent. A possible explanation for this discrepancy is that while the Lens seedlings for the second series of experiments were obtained from the same commercial source it was not ascertained that they were the same strain as those used previously.

The epidermal cell frequencies of the control plants whilst showing the reasonable agreement among themselves are not in close accord with those plants for which they were intended as controls. Perhaps this can be attributed to the fact that only about half the number of determinations were made.

A further point of interest centres in the production of lateral roots. These were very much better developed in the roots transferred to air than

in those kept in 10 per cent. carbon dioxide. Of the former it is interesting to note that the laterals appear to be approximately equally well developed in the shrivelled roots as in those which continued their elongation. If the production of lateral roots be regarded as some measure of the decrease in the activity of the root-apex this suggests that the rapid elongation of the primary root which follows the transfer is not a true increase in the growth rate. It is perhaps analogous to the elongation of an etiolated stem. This region is always of smaller diameter than the hairy regions above and below it, and although the epidermal cells are very long they are narrower than the cells of hair-bearing regions.

In this way, therefore, the development of root-hairs may become discontinuous as a result of changes in the environment of the root, and it will now be appreciated why emphasis was laid on the choice of roots showing a regular presence or absence of root-hairs. It was noted in many instances that when hairy and non-hairy zones alternated on one root the relationship between hairiness and the development of the epidermal cells, which has been described in an earlier part of this paper, no longer held good but became quite irregular. The temporary cessation of hair production in *Lens* when the radicles penetrate the soil surface (noted above) is explicable in this way. It seems probable, too, that the unexplained tufts of hairs observed by Snow in her experiments developed as an accompaniment of an earlier retardation.

The discontinuity arises from the fact that the root-hair is a lateral outgrowth of the otherwise (almost) mature epidermal cell, and its development takes place some distance behind the root-tip. Any change in environment will, therefore, affect differently the epidermal cells and the hairs, and there will necessarily be a time-lag before these structures can regain their normal equilibrium. It is the failure fully to appreciate this point that has led to the somewhat chaotic results of the past, and if due allowance be made for this inevitable lag the connexion between root-hair production and the development of the epidermis becomes more intelligible.

#### SUMMARY.

Root-hair formation has been regarded either (*a*) as stimulated by retardation of the longitudinal growth of the root, or (*b*) as an accompaniment of optimum conditions of growth and not a consequence of a retardation.

It is here shown by observation and experiment on a number of species that if the roots observed are consistently hairy or non-hairy and do not exhibit alternation from one condition to the other, the production of root-hairs is accompanied by an increase in the dimensions of all the cells of the

piliferous layer. This may be regarded as further evidence in support of the view (b), above.

It is also of importance in another direction in that it invalidates root-hair frequency as a measure of the intensity of root-hair formation, and a new criterion, the root-hair index, derived by analogy with the stomatal index, is put forward. This is applied in various ways, particularly in using the transfer of seedlings from one medium to another so as to gauge the effect of deterioration and amelioration of conditions of growth on root-hair development.

These experiments are discussed in relation to analogous ones carried out by others, and it is pointed out that as the root-hair is a lateral outgrowth of an otherwise almost mature epidermal cell, which develops some distance behind the root-tip, any change of environment must for a time exert a differential effect on the rate of growth of the root as a whole and on the production of root-hairs. Its effect cannot, therefore, be fully described until the root has come into equilibrium with the new conditions, and previous conclusions which took no account of this point must be regarded as inadmissible.

The use of the root-hair index, and a due recognition of the time-lag between a change in conditions and the completion of the reaction to it of the root, permits the formulation of a logical account of the relationship between the development of the epidermis and the root-hairs and explains some of the earlier divergent results.

A number of calculations have also been made relating to the increase in the superficial area of the root surface brought about by root-hair formation, and the possible significance of this increase is discussed.

In conclusion, the writer would like to express his keen appreciation of the helpful advice and criticism given to him by Professor E. J. Salisbury, under whose direction this work was carried out.

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# The Effect of Light on Transpiration.

BY

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IT is well known that leaves transpire more in light than in darkness, and it is a generally accepted notion that this is due, at least in part, to some physiological response to light independently of temperature effects. While studying factors which affect yield of wheat I had occasion to review literature on the effects of light on plant growth, and in doing so came to the opinion that in relation to the effect of light on transpiration, insufficient attention has been given to associated increase of leaf temperature, and to the effect of such increased temperature on evaporation.

Careful attention to the first of these effects has been given by Henderson<sup>1</sup> who, to avoid the complications introduced by stomatal movements, experimented on mesophyll exposed by slitting the leaf. Experiments were performed in a controlled environment chamber. It was observed that the increases of transpiration rate due to light of 200 candle-power 3 ft. distant from the leaves varied between zero and 8 per cent. The leaf temperature rose on illumination by an amount varying between 0° and 1°C. Whence the author concluded: 'From the foregoing data for Ivy, in the absence of stomatal control, it will be clear that light has not the marked effect on water-loss from mesophyll that has been claimed. In the preliminary series of experiments a slight increase of the order of 5 per cent. is found, however, and . . ., under the conditions of the experiments, the increased temperature would only bring about an increase of transpiration of about 1 per cent. The interesting result therefore emerges that, apart from the heat effect, there is a slight direct effect of light on the mesophyll leading under the conditions of the experiments to an increase of water-loss of about 4 per cent.'

The air in the experimental chamber was being continually changed. For evaporation in a current of air the following formula is applicable<sup>2</sup>:

$$V = (0.031 + 0.0135W) (p_s - p_o) 760/P$$

where  $V$  is rate of evaporation (kg. per sq. m. per hr.),

<sup>1</sup> F. Y. Henderson, *Ann. Bot.*, xl. 507-34, 1926.

<sup>2</sup> Critical Tables, v. 53-5.



$W$  is velocity of the air current over the evaporating surface (m. per sec.),

$P$  is atmospheric pressure (mm.),

$p_s$  is saturation vapour pressure (mm.) at the temperature of the evaporating pressure,

$p_o$  is vapour pressure (mm.) in the surrounding air.

For the conditions of the experiments  $W$  and  $P$  may be considered constant, whence the relative increase in evaporation due to a change of temperature of the evaporating surface, accompanied by changes of vapour pressure in the surrounding air, is given by

$$\frac{V_2 - V_1}{V_1} = \frac{p_{s2} - p_{o2}}{p_{s1} - p_{o1}} - 1,$$

where  $V_1$  and  $V_2$  are the values of  $V$  at the initial and final temperatures respectively, and  $p_{o1}$ ,  $p_{o2}$ ,  $p_{s1}$ , and  $p_{s2}$  are the corresponding values of  $p_o$  and  $p_s$ .<sup>1</sup>

The sensitivity of evaporation rate to small changes of temperature and/or humidity should be noted. For example, starting with 70 per cent. relative humidity and with both air and evaporating surface at 23° C., if the temperature of the evaporating surface be raised to 24° C. while *vapour pressure* in the air ( $p_o$ ) remains constant, evaporation would increase by 20.5 per cent.; if, however, vapour in the air be simultaneously increased to maintain a relative humidity of 70 per cent. at 24° C., evaporation would increase by only 6.2 per cent. This explains (apart from errors in technique as noted by Henderson) why the increase of transpiration rates observed by Henderson was less than that observed by other workers who did not simultaneously increase the vapour pressure in the air.

Henderson gives detailed data for four experiments with Ivy leaves exposed alternately in darkness and to steady light. These data are summarized in Table I, along with derived data used in order to apply the above formula. The last two columns show the observed changes in rate of water-loss, and the changes of evaporation rate to be expected as due to the observed changes of temperature. In comparing these figures several sources of error should be borne in mind. *Firstly*, the equation for estimating expected evaporation is empirical and cannot be trusted to give exact estimates in any particular case. *Secondly*, observations of humidity during the experiments have not been published, and it has been assumed that relative humidity was constant at 70 per cent.<sup>2</sup> *Thirdly*, temperature was changing during the period of observation and was observed at only

<sup>1</sup> Other formulae for estimating evaporation rates (see, for example, Critical Tables, loc. cit., and Maximov, The Plant in Relation to Water) lead to either identical or approximately equal values for relative increases whether the air be moving or still.

<sup>2</sup> If relative humidity were not constant it seems probable that, in these experiments, any change would be towards a lower relative humidity on account of some lag in the ability of the apparatus to

TABLE I.

Data from	Leaf temp. (° C.).		Air temp. (° C.).		$p_s$ (mm.). <sup>1</sup>		$p_o$ (mm.). <sup>1</sup>		Water loss mg. in 30 min.		Observed difference (per cent.).	Expected difference (per cent.).
	Dark.	Light.	Dark.	Light.	Dark.	Light.	Dark.	Light.	Dark.	Light.		
Table I	22.7	23.8	22.9	23.6	20.71	22.13	14.67	15.31	28.0	29.0	3.6	12.8
" 1 <sup>2</sup>	23.1	23.9	23.5	23.5	21.22	22.26	15.21	15.21	27.0	28.5	5.6	17.3
" 2	23.3	23.6	24.0	24.2	21.47	21.87	15.68	15.87	84	87	3.6	3.6
" 3	23.8	24.8	23.8	24.1	22.13	23.50	15.49	15.77	47.5	52	9.5	16.3
" 4	24.3	25.2	24.2	25.0	22.80	24.06	15.87	16.65	50	54	8.0	6.9

<sup>1</sup> From Smithsonian Meteorological Tables, Fifth Edition (p. 170), 1931. For  $p_o$  relative humidity is assumed to have been constant at 70 per cent.  
<sup>2</sup> Change from light to dark. Other observations are on dark to light.

a few instants. An approximate average temperature, estimated from the trend of the graphs, has been used in the calculations. *Fourthly*, there are unavoidable experimental errors in observing the increased water-loss. Arguments may be advanced in favour of (1) taking the average rate for each experiment over the whole period in each condition (darkness and light), or (2) comparing only the last half-hour in darkness with the second half-hour in light. The latter comparison shows the greater differences and has been used for the summary of the data shown in Table I. Of the two alternatives it is the more favourable to the accepted belief which is here questioned. *Fifthly*, the observed leaf temperatures do not give the temperature of the evaporating *surface*. This is appreciably lower both in darkness and in light. However, if it may be assumed that the *change* of temperature is about the same, calculations based on internal leaf temperature are not appreciably in error.<sup>1</sup> For these reasons close agreement between observed and expected changes of evaporation rate is not to be expected, and the agreement shown by Table I may be considered to be reasonably good. Expected increases are, on the average, greater than observed increases. It seems therefore safe to conclude that there is no evidence that water-loss (assumed by Henderson to be equivalent to, or comparable with, transpiration) was accelerated on exposure to light more than may have been caused by the heating effect.

In Tables VI and VII of Henderson's paper data are given for rates of water-loss with varying light intensities. The relative rates of water-loss observed are shown in Tables II and III herewith, where they are compared to expected

TABLE II.  
(Corresponding to Henderson's Table VI.)

Light intensity thermopile deflexion (cm.).	Temperature (° C.).		Relative humidity.	Relative water-loss	
	Air.	Leaf.		Observed.	Expected.
(Dark) 0	26.6	26.4	55	100	—
8.3	26.6	26.4	55	100	100
13.3	26.8	26.4	56	98.3	96.2
19.3	26.4	26.8	55	101.7	106.9
25.3	26.8	26.7	55	109.2	102.5
34.3	26.5	26.7	56	110	102.5
37.3	26.6	26.8	55	108.3	105.4

rates calculated as above. The agreement between observed and expected

supply the greater quantity of water vapour necessary to maintain the same relative humidity at a higher temperature. We are therefore more likely to under-estimate than to over-estimate the increase of evaporation due to rising temperature.

<sup>1</sup> For example, if evaporating surface and atmosphere both change from 23° to 24° C. the estimated increase of evaporation would be 6.2 per cent.; if the evaporating surface change from 22° to 23° C. under otherwise similar conditions it would be 6.3 per cent.

TABLE III.

(Corresponding to Henderson's Table VII.)

Light intensity thermopile deflexion (cm.).	Temperature (° C.).		Relative humidity.	Relative water-loss	
	Air.	Leaf.		Observed.	Expected.
(Dark) 0	25.0	24.8	61	100	—
8.5	25.0	24.7	(61)	100	98.4
13.5	25.0	24.8	(61)	101.6	100
17.5	25.2	24.8	60	100	100.8
21.5	25.0	25.0	(60)	103.1	105.8
25.5	25.2	24.8	60	104.7	100.8
29.5	25.0	25.0	59	106.2	108.4
40.5	25.4	25.1	(59)	106.2	106.4
33.5	25.4	25.5	59	106.2	112.8
(Dark) -0.5	25.2	25.2	59	101.6	109.7 <sup>1</sup>

The figures in brackets in column 4 are assumed figures.

values is again reasonably good. These experiments therefore support the same conclusion.

It is indicated by the author that there was no increase of water-loss from a 'Piche' atmometer over the range of light intensities studied. If this apparatus was made with a white filter paper, its temperature would be expected to be less susceptible to influence by low light intensities than would the temperature of a green leaf.

The results obtained with intermittent light of varying frequency are interesting. Leaf temperatures, however, are not given for these experiments. They therefore fall outside the scope of the present critical analysis.

# SUMMARY.

Examination of the data of Henderson (*Ann. Bot.*, xl. 507-34, 1926), who concluded that light had a direct effect on transpiration apart from any heating effect or effect on stomatal aperture, leads to the view that the increased transpiration observed (of the order of 4 per cent.) may in fact have been due to the small rise in temperature of the leaves resulting from the absorption of radiant energy.

I am grateful to Mr. A. J. Higgs, of the Commonwealth Observatory, Mt. Stromlo, for verifying the calculations.

<sup>1</sup> Probably over-estimated, as on return to darkness the surface would cool faster than the interior of the leaf.



# Further Studies on Transport in the Cotton Plant.

## IV. On the Simultaneous Movement of Solutes in Opposite Directions through the Phloem.<sup>1</sup>

BY

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With six Figures in the Text.

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### I. INTRODUCTION.

IT would appear that plant physiologists are not yet agreed as to the *type* of mechanism involved in the longitudinal transport of materials through the sieve-tube. Münch (8) has elaborated a theory involving a directed mass flow of solution, while others (1, 4) favour a movement of solute rather than solution and stress (2) the resemblance between the gross phenomena of transport and diffusion. The *Druckstrom* theory possesses a completeness and simplicity that has much to recommend it, while the theory of *activated diffusion* is rather vague concerning the method whereby diffusion through the sieve-tube is accelerated. As protoplasmic streaming has apparently to be rejected as a method of accelerating diffusion, and as respiration appears to be a factor in transport, the suggestion (7) has been

<sup>1</sup> Paper No. 13 from the Physiological Department of the Cotton Research Station, Trinidad.  
[*Annals of Botany*, Vol. L. No. CXCVII, January, 1936.]

made that the energy released in respiration is in some way utilized in reducing the resistance of the solvent.

In an earlier paper (6) from this Station, the possibility of sugar and nitrogen travelling through the sieve-tubes in reverse directions was discussed. If solutes can move in opposite directions, any theory involving a directed mass flow of solution becomes untenable, unless both types of movement are possible, a mass flow through the vacuole and movement of solutes through the cytoplasm.

The attempt was made to make sugar and nitrogen travel in reverse directions in three different ways. First, we tried, by darkening a number of leaves with bags, to show that sugar could enter the lamina while nitrogen was being exported from it. At the time this experiment was made the function of the transition cell (cf. 9) was not appreciated, and the failure of the experiment was doubtless due to the difficulty with which sugar moves from the fine veins into the lamina. In a second experiment we attempted to determine whether the nitrogen absorbed by the root could travel *up* the phloem while sugar was travelling downwards. We were unable to demonstrate upward movement of nitrogen from the root in the phloem. Failure of nitrogen to move up the phloem from the root was due, perhaps in part, to the difference in the relative dimensions of companion cell and sieve-tube in root and leaf. We next arranged an experiment to see if nitrogen could travel from the older basal leaves up the phloem to the younger apical tissues at the same time that sugar was travelling downwards. This attempt was successful in that nitrogen did travel up the stem from the basal leaves while sugar was descending in the bark, but the possibility of downward movement of nitrogen in the phloem from the basal leaves to the root and upward movement thence in the xylem to the young tissues at the apex was not excluded. As this line of approach appeared the most promising we have repeated this experiment with certain modifications, and the results are presented in the present paper.

## II. EXPERIMENTAL REVERSAL OF MOVEMENT.

### (a) *Procedure.*

The plants were grown in water culture in two glass-houses, which will be termed houses A and B respectively. A complete culture solution was used for the first two and a half months, after which the complete solution was replaced by one containing only calcium and iron. The solutions were renewed twice a week. The object of the omission of nitrogen, phosphorus, &c., that is, the phloem-mobile elements, from the solution, was to cut off the normal root supply of these elements to the young apical tissues, and thus ensure that there was upward movement only from the reserves stored in the basal region. To assist downward movement of

sugar from the apical to the basal region (see Fig. 1), the leaves of the basal region were covered with paper bags. The paper was black and glazed on the inside. This bagging of the leaves should also, it was thought, assist in the export of nitrogen from the leaves.

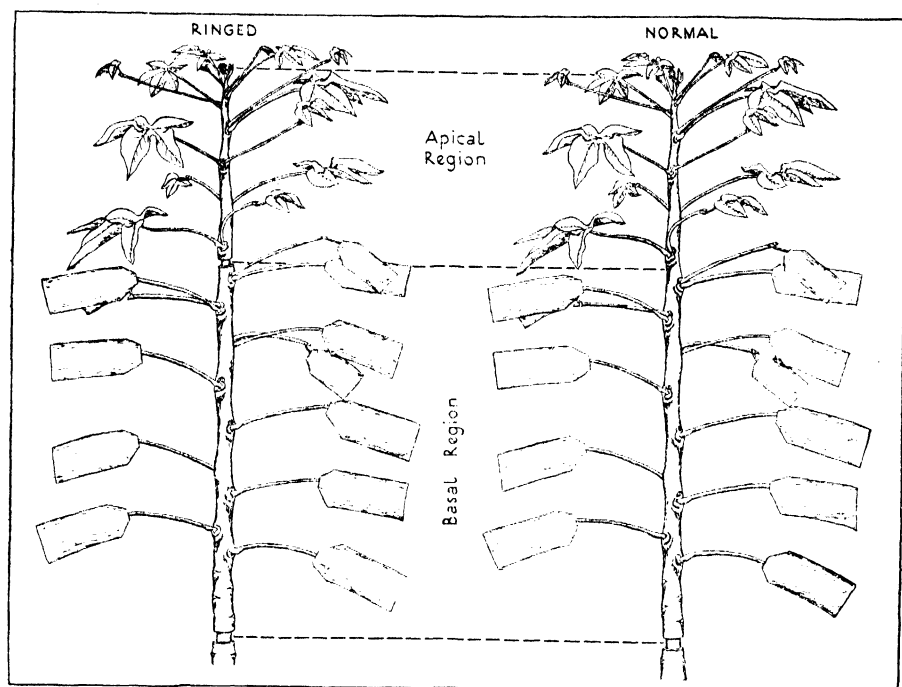


FIG. 1. Showing treatment of ringed (left) and normal (right) groups.

The plants in house A were on the average approximately 61 cm., and in house B 57.5 cm. in height. They consisted of the main axis, the leaves on the main axis and the fruiting-branches. Each fruiting-branch consisted of a single internode and a single leaf. In order to define the channel, bark or wood, in which materials moved, the following procedure was adopted. To ensure that nitrogen did not travel downwards from the basal leaves to the root in the phloem and thence upwards in the wood to the apical tissues, all plants were ringed in the internode above the cotyledonary node. To determine the channel of upward movement of nitrogen and downward movement of sugar, a ring was made in one group of plants between the apical and basal regions.

There were thus two groups of plants: a Normal group with one ring, and a Ringed group with two rings. The apical region stretched from the apical bud to the internode between the seventh and eighth nodes from the apex, and the basal extended downwards to the ring above the cotyledonary



node, while the region below the lower ring is referred to as the root region. Immediately before collection, an additional ring was made on the Initial and Normal plants to correspond with the upper ring of the Ringed group. The plants were graded on the basis of height and total number of nodes. In house A ten plants and in house B nine plants were sampled at each collection. There was only one sample taken at each collection except at the Initial collection, when two samples were taken. *The results throughout are expressed on the sample basis, and represent the weights of material in ten plants.*

The sequence of events was as follows :

*Time-table.*

November 9, 1933. Full culture solution replaced by one containing only calcium and iron.

November 10, 1933. Ringing of stem, bagging of leaves, and Initial collection.

November 13, 15, 17, 20, 22, and 24, 1933. Collections of Normal and Ringed groups.

(b) *Results.*

(1) *Nitrogen.* The results for the apical region are shown graphically in Fig. 2. The actual weights of nitrogen found in the samples and the regression lines of nitrogen on time are shown in the upper part of the figure. The *distribution* of nitrogen between the apical and basal regions is shown in the lower part by expressing the weight of nitrogen in the apical region as a percentage of that in the combined apical and basal regions.

Taking first the results for house A, it will be seen that nitrogen moved into the apical regions of both the Normal and the Ringed groups. The correlation coefficient (Normal  $r = +0.92$  ; Ringed  $r = +0.76$ ) between nitrogen content and time is statistically significant for each group. The increase during the course of the experiment was, however, about three times as great in the Normal as in the Ringed group. The percentage increases, calculated from the regressions, during the course of the experiment were 34.8 for the Normal and 11.8 for the Ringed group. The question at issue is to what this difference is due. The nitrogen that moved into the Ringed group must have travelled upwards in the wood. The excess<sup>1</sup> (or part of it) exhibited by the Normal group might have travelled via the phloem, in which case upward movement of nitrogen via

<sup>1</sup> It is probable that leakage of nitrogen into the vessels was less in the Normal than in the Ringed group, for the sugar concentration in the latter must have been smaller than in the former. Furthermore, the concentration of non-protein N in the wood parenchyma of the Ringed group would be greater than in the Normal.

the phloem would be demonstrated, or might have travelled upwards in the wood, the difference between the two groups being due to damage to the wood as a result of the operation of ringing. The latter hypothesis

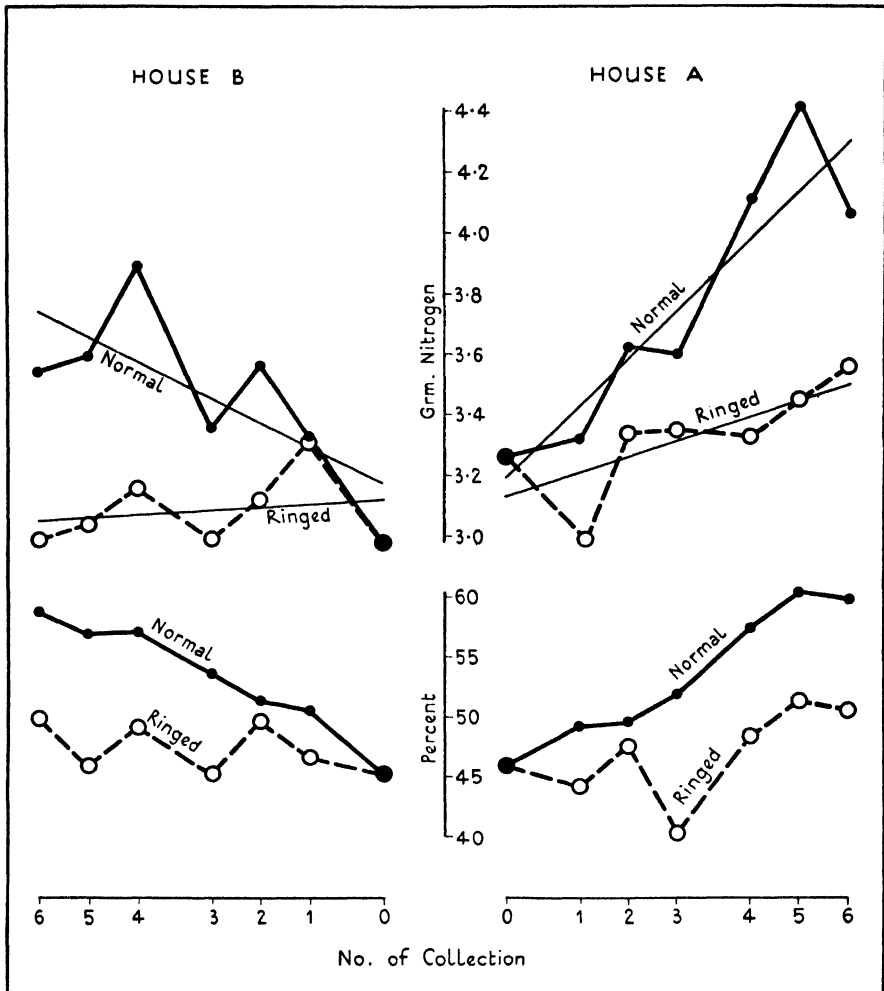


FIG. 2. Showing actual weights of nitrogen in apical region (upper) with regression lines of nitrogen on time, and weights in apical region as percentages of combined weights in apical and basal regions (lower).

assumes a very great reduction in the volume of water moving upwards through the wood as a result of ringing. As the leaves of the Ringed group remained fully turgid, such a reduction in the rate of transpiration seems unlikely.

As the nitrogen supply to the roots was curtailed, nitrogen entering the apical region via the wood must have leaked into the vessels from the

adjacent wood parenchyma of the basal region. Calculation of the reduction in the volume of water entering the apical region of the Ringed group is possible if the assumption is made that the gradient of non-protein nitrogen between parenchyma and vessel determines the rate of this leakage, and if we know the concentrations of non-protein nitrogen in parenchyma and vessel. A conservative estimate of the gradient of non-protein nitrogen between parenchyma and vessel of the normal plant would be 90 mg. per 100 gm. water; 100 mg. for the parenchyma (cf. 3) and 10 mg. per 100 gm. water for the vessel (cf. 5). Since approximately three times as much nitrogen entered the apical region of the Normal as entered the corresponding region of the Ringed group, the gradient into the vessels of the Ringed group must be reduced from 90 to 30 mg. non-protein nitrogen. As this change in gradient arises by hypothesis as the result of a slowing up in the rate of the transpiration stream, the change in gradient must be due to a rise in the concentration in the vessel sap from 10 to 70 mg. per 100 gm. water. A sevenfold increase in the concentration of the vessel sap would involve an even greater reduction in the rate of the transpiration stream as a result of ringing. As the leaves remained fully turgid in the Ringed group such a reduction in transpiration would seem unlikely.

The results for the apical region of house B differ in that there was little or no movement of nitrogen into the Ringed group. The correlation coefficient in this group between nitrogen content and time is in fact negative ( $r = -0.22$ ), but it is not significant. If there was upward movement through the wood in the Ringed group, it must have been lost into the atmosphere. In the Normal group there was a considerable import of nitrogen. The correlation coefficient with time is positive ( $r = +0.73$ ) and partially significant. In this case, there are therefore good grounds for believing that nitrogen ascended the stem in the phloem of the Normal group. Upward transport through the wood is not indicated.

The results for the basal region are shown in Fig. 3. In both houses and in both groups there were losses in nitrogen; the losses being much greater from the Normal than from the Ringed group.

The changes, both actual and relative, in the weights of nitrogen during the whole course of the experiment in the various regions of the plant and in the Whole Plant are shown in Table I. These values are calculated from the regression lines, which are assumed to be linear. For the apical plus basal regions and for the Whole Plant, the changes can also be calculated by summing the changes in the component parts; these values are shown in italics immediately below the regression values. This table also shows the correlation coefficients between the weights of nitrogen in the various regions and time. Statistically significant ( $P = 0.05$ ) coefficients are shown in italics and partially significant ( $P = 0.10$ ) ones in heavy type.

In both houses the Whole Plant appears to have lost nitrogen, the losses in house B exceeding those in house A. In both houses very marked losses are shown by the root, these being greater from the Ringed than from the Normal group. These differences are not, however, significant. The

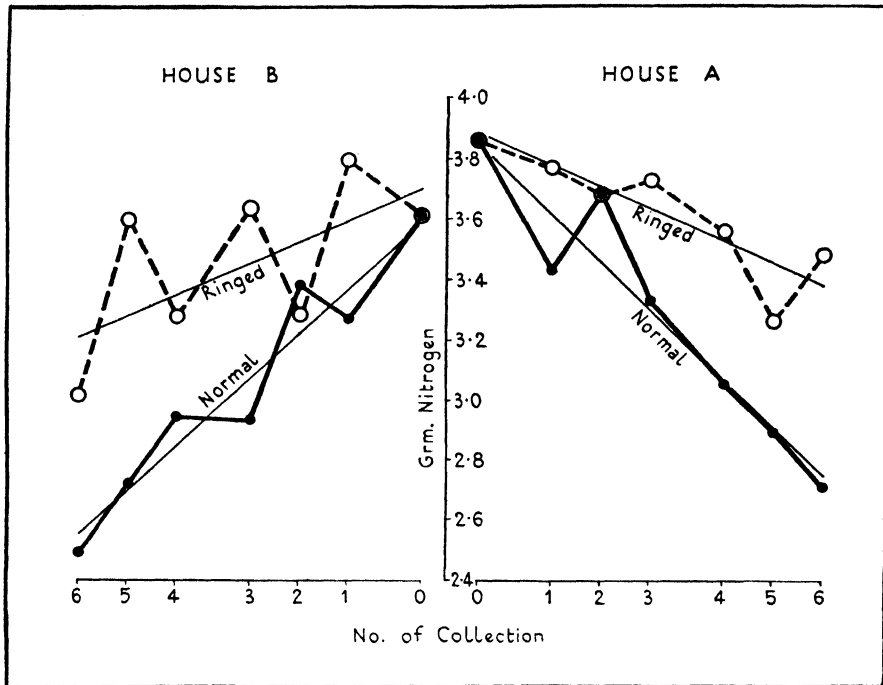


FIG. 3. Showing weights of nitrogen in basal region with regression lines of nitrogen on time.

losses from the root were presumably into the culture solution, as the apical plus basal region in no case registers a gain.

In house A, the apical plus basal regions show little change and the bulk (Ringed group) if not the whole (Normal group) of the nitrogen lost from the basal region appears in the apical region. As the loss by the basal region of the Ringed group exceeded the gain by the apical region, there is a suggestion of loss into the atmosphere. This suggestion is reinforced by the results for house B, for in both groups the losses from the basal region exceeded the changes in the apical region.

To sum up, losses of nitrogen from the root and from the basal region<sup>1</sup> into the culture solution or atmosphere respectively are indicated in both houses. In house A, there was some movement from the basal region of the Ringed group via the wood to the apical region. The gain by the apical region of the Normal group was, however, about three times as great. It

<sup>1</sup> A loss into the culture solution from the root might be expected as a result of carbohydrate starvation, and into the atmosphere from the basal region owing to the bagging of the leaves.

is concluded that these differences must, at least in part, be due to upward transport in the phloem. In house B, there is no evidence of movement from the basal to the apical region of the Ringed group via the wood. In the

TABLE I.

*Actual (gm.) and Relative (per cent.) Changes in Nitrogen during the Experiment, Calculated from Regressions, in Regions and in Whole Plant, with Correlation Coefficients.*

House A.						
		Apical.	Basal.	Apical plus basal.	Root.	Whole Plant.
Normal	Actual	+1.109	-1.109	-0.004 0.000	-0.225	-0.228 -0.225
	Relative	+34.8	-28.8	-0.1	-16.2	-2.7
Ringed	Actual	+0.370	-0.505	-0.115 -0.135	-0.303	-0.403 -0.438
	Relative	+11.8	-13.0	-1.6	-21.9	-4.8
Correlation coefficients	Normal	+0.92	-0.96	-0.01	-0.59	-0.26
	Ringed	+0.76	-0.87	-0.25	-0.82	-0.56
House B.						
Normal	Actual	+0.572	-1.042	-0.470 -0.470	-0.246	-0.706 -0.716
	Relative	+18.1	-29.0	-7.0	-19.9	-8.8
Ringed	Actual	-0.074	-0.470	-0.538 -0.544	-0.289	-0.874 -0.833
	Relative	-2.4	-12.7	-7.9	-23.2	-10.8
Correlated coefficients	Normal	+0.73	-0.96	-0.53	-0.69	-0.61
	Ringed	-0.22	-0.62	-0.59	-0.93	-0.75

Normal group, however, a marked movement of nitrogen took place from the basal region to the apical region, and it is concluded that this upward movement must have occurred mainly in the phloem.

(2) *Carbohydrate.* The dry weights of the apical region are shown in Fig. 4. In the lower part of the graph are shown the actual dry weights, while in the upper the dry weight of the apical region is expressed as a percentage of the dry weight of the combined apical and basal regions. The actual dry weights do not show any conspicuous differences between the Normal and Ringed groups. It is true that the mean values for the Ringed group exceed the mean values for the Normal group in both houses, but the differences are not statistically significant (Table II).

In the upper part of the figure, the differences in the *distribution* of dry weight are in both houses in favour of the Ringed group, and in both houses the differences between the mean values for the two treatments are fully significant (Table II). Downward movement of carbohydrate is

therefore suggested by consideration of the changes in the distribution of dry weight between the apical and basal regions.

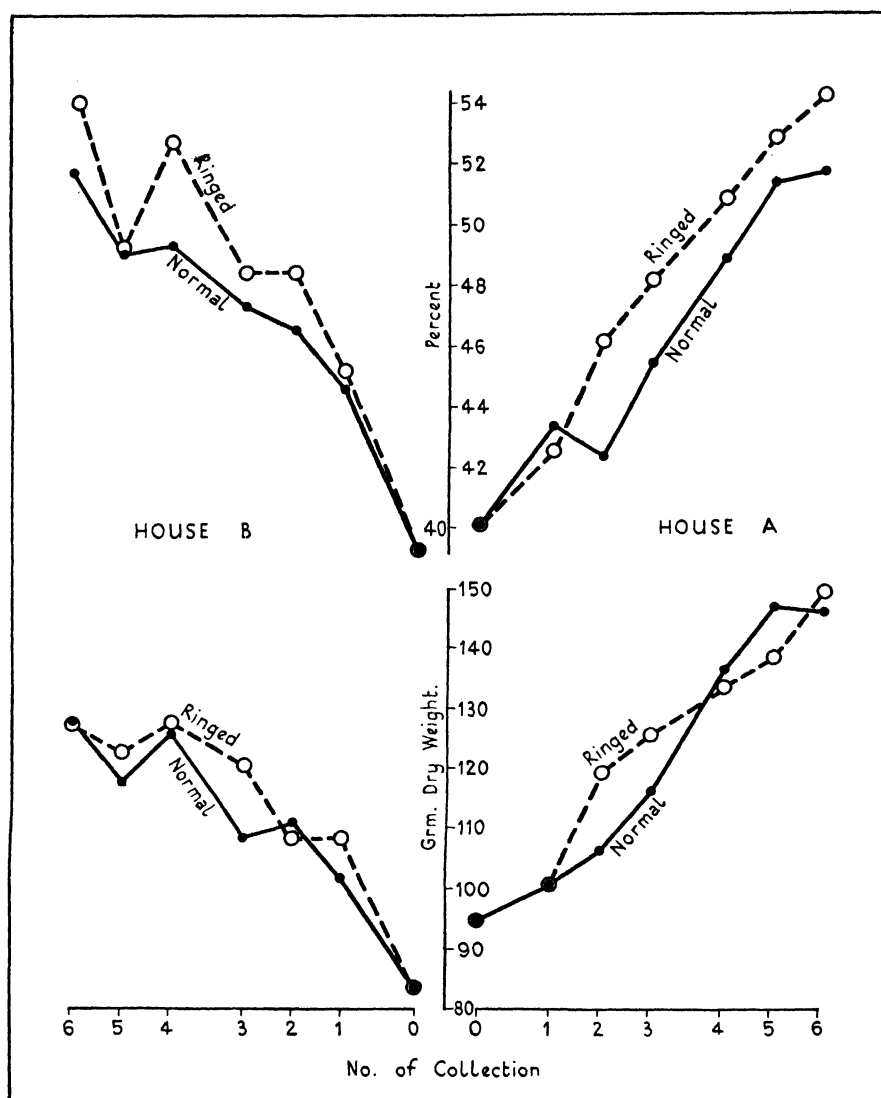


FIG. 4. Showing actual dry weights in apical region (lower) and dry weights in apical region as percentages of combined dry weights in apical and basal regions (upper).

As the Normal group contained a greater weight of nitrogen than the Ringed, and as an appreciable part of the dry weight must consist of nitrogenous substances, the difference in favour of the Ringed group will be increased by making allowance for the difference in the nitrogen content of

the two groups. We have on previous occasions (5) taken the weight of carbohydrate to be equal to the dry weight from which is deducted 5.7



FIG. 5. Showing actual weights of carbohydrate in apical region (lower) and weights in apical region as percentages of combined weights in apical and basal regions (upper).

times the weight of nitrogen. Adopting this procedure, which though arbitrary is nevertheless unlikely to greatly overestimate the differences in the weights of carbohydrate between the two groups, we can calculate the values

for *apparent* carbohydrate. It must be realized further, that not only nitrogen, but phosphorus, and probably all the phloem-mobile mineral elements,

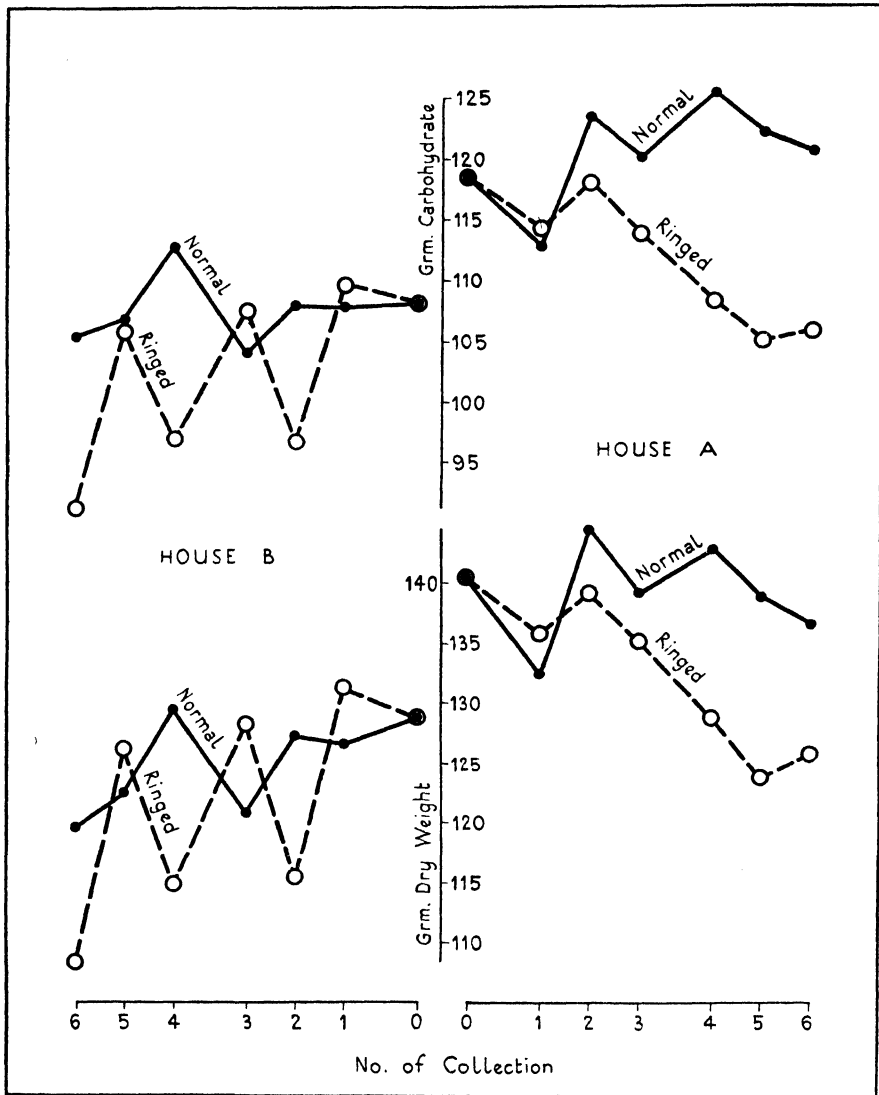


FIG. 6. Showing dry weights (lower) and weights of carbohydrate (upper) in basal region.

were present in greater amount in the Normal than in the Ringed group, and that these differences would emphasize the carbohydrate differences in favour of the Ringed group.

The results for carbohydrate in the apical region are shown in Fig. 5. It will be seen that the differences between the two groups are now much



more marked. The mean differences between the two groups for the actual values and for the values for the apical as percentages of the apical plus basal regions are shown in Table III. The results indicate that carbohydrate

TABLE II.

*Mean Dry Weight Values for Apical Region of Ringed and Normal Groups, and these Values as Percentages of Combined Dry Weights of Apical and Basal Regions.*

House	Actual (gm.).		Actual as percentage of apical plus basal.	
	A.	B.	A.	B.
Ringed	127.96	119.03	49.34	49.64
Normal	125.47	115.39	47.45	48.12
Difference ( $R-N$ )	2.49	3.64	1.89	1.52
Significant differences	$P = 0.05$	8.38	5.57	1.57
	$P = 0.10$	6.57	4.37	1.23
				0.98

TABLE III.

*Mean Carbohydrate Values for Apical Region of Ringed and Normal Groups, and these Values as Percentages of Combined Carbohydrate of Apical and Basal Regions.*

House	Actual (gm.).		Actual as percentage of apical plus basal.	
	A.	B.	A.	B.
Ringed	108.94	101.34	49.54	50.03
Normal	103.51	95.17	46.14	46.94
Difference ( $R-N$ )	5.43	6.17	3.40	3.09
Significant differences	$P = 0.05$	6.82	5.19	1.79
	$P = 0.10$	5.34	4.07	1.40
				1.16

TABLE IV.

*Mean Dry Weight and Carbohydrate Values for Ringed and Normal Groups in Basal Region.*

House	Dry weight.		Carbohydrate.	
	A.	B.	A.	B.
Normal	138.96	124.40	120.83	107.57
Ringed	131.37	120.76	110.98	101.21
Difference ( $N-R$ )	7.59	3.64	9.85	6.36
Significant differences	$P = 0.05$	4.74	10.46	3.93
	$P = 0.10$	3.64	8.20	3.02
				7.17

has travelled downwards in the phloem from the apical to the basal region of the Normal group.

The dry weight and carbohydrate results for the basal region are shown

in Fig. 6. As the leaves in the basal region were covered with paper bags, well-marked losses in dry weight and carbohydrate are shown by the Ringed group in both houses. The mean differences are shown in Table IV, and are, it will be seen, fully significant in house A for dry weight and for carbohydrate.

The downward movement of carbohydrate in the phloem suggested by an examination of the results for the apical region is thus corroborated by the results for the basal region.

### III. SUMMARY.

1. Cotton plants were grown in water culture for two and a half months with a full nutrient solution. At the end of this period, all nutrients except calcium and iron were omitted from the solution. The plants were thus starved of the phloem-mobile elements nitrogen, phosphorus, potassium, &c.

2. At the beginning of the experiment, which lasted for fourteen days, the plants consisted of the root and the main axis with its leaves. The main axis was divided into an apical and a basal region (see Fig. 1).

3. There were two groups of plants, both of which were ringed below the basal region. In one group, an additional ring was made between the apical and basal regions. The former was termed the Normal and the latter the Ringed group. The basal leaves of both groups were covered with paper bags with a view to arresting photosynthesis.

4. It was found that nitrogen travelled upwards in considerable amounts from the basal to the apical region of the Normal group, while little or none entered the apical region of the Ringed group. It is inferred that some at least of the nitrogen that entered the apical region of the Normal group travelled upwards in the phloem.

5. It was also found that carbohydrate travelled downwards in the phloem from the apical region of the Normal group and entered the basal region.

6. It is concluded that nitrogen and carbohydrate may travel simultaneously in opposite directions through the phloem.

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# The Interaction of Factors in the Growth of Lemna.

## VII. The Effect of Potassium on Growth and Multiplication.

BY

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With seven Figures in the Text.

### INTRODUCTION.

SINCE 1927 *Lemna minor* has been used in this laboratory (see 1) for a study under controlled conditions of the interaction of factors, and especially of the interaction of light intensity with other factors.

The aim of the work to be presented in a series of papers, of which this is the first, is the study of the interaction of light intensity with the nitrogen and the potassium content of the nutrient solution. At the outset of the work it was obviously advisable to study in detail the effect of varying a single factor. The present paper is a study of response to varying the potassium factor under constant light intensity.

### EXPERIMENTAL PROCEDURE.

*Nutrient solution.* It was found useful to vary the solution from that of Clark (2), which had been previously used in this laboratory. The new solution has the following composition :

CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	0.101 gm.
Ca(NO <sub>3</sub> ) <sub>2</sub> , 4H <sub>2</sub> O	0.581 "
Mg(NO <sub>3</sub> ) <sub>2</sub> , 6H <sub>2</sub> O	0.382 "
MgSO <sub>4</sub>	0.255 "
K <sub>2</sub> SO <sub>4</sub>	0.668 "
Fe <sub>2</sub> Cl <sub>6</sub>	0.002 "
Water	1000 c.c.

This solution possesses the advantage that omission of either nitrate or potassium leaves the plant colonies supplied with sufficiency of the ions (Ca, Mg, and SO<sub>4</sub>) of the salts in which nitrate and potassium are supplied. The assumption has been made throughout that any changes observed are due to the omission of nitrate and potassium and not to changes in the concentration of Ca, Mg, and SO<sub>4</sub>.

[Annals of Botany, Vol. L. No. CXCVII. January, 1936.]

*Measurement of growth and calculation of errors.* The main measures of growth are counts of frond number and estimates of frond area and of dry weight by a technique previously described (1, 6).

Standard errors attached to the indices of relative-frond-number-increase are derived from daily counts, thus assuming that day to day variation is uncorrelated. This assumption is probably unwarranted, so the errors may be overestimated, but considerations of space and labour precluded duplicate colonies.<sup>1</sup>

From time to time estimates of the errors of sampling of area and dry weight have been made. Table I gives the summarized results of three such estimations. In all experiments the variance as between pairs of duplicates appears to be greatly exceeded by the extent of daily fluctuation, showing that the methods of sampling used were more than adequate for the accuracy of the growth technique available.

TABLE I.  
*Errors of Sampling.*

The percentage errors given are the standard deviation of one observation and of the mean of two observations.

Number of parallel samples.	Number of fronds per sample.	Average frond area (sq. cm.).		Average frond dry weight (mg.).		Dry weight per unit area (mg. per sq. cm.).	
			% %		% %		% %
7.	17-20	0.0408 ± 3.6,	2.6	0.084 ± 7.0,	5.0	2.06 ± 3.6,	2.6
9.	16	0.0422 ± 3.6,	2.6	0.075 ± 5.3,	3.8	1.78 ± 2.2,	1.6
8.	12	0.0594 ± 4.2,	3.0	0.091 ± 5.5,	3.9	1.54 ± 3.3,	2.4
		Means	3.8, 2.7		5.9, 4.2		3.0, 2.2

*Experimental conditions.* The plant colonies were grown in silica beakers (150 c.c.) placed on a movable shelf in a glass-topped case. Above the case was suspended a 1,000-watt Mazda gas-filled lamp, the heat rays being cut off by a trough of running water. Light intensity, as estimated by a Holophane lumeter, was maintained at 450 foot-candles. The case was situated in a darkened room with the temperature maintained at 25° C. by three electrically heated radiators, controlled by a mercury thermo-regulator. A continuous current of air drawn from outside the laboratory and heated to 25° C. was blown through the case by a water-blower. The nutrient solution was changed and the beakers cleaned, daily. The number of fronds was counted daily and samples withdrawn every second day for estimates of area and dry weight. Allowance is made

<sup>1</sup> The use of the standard error of day to day variation brings out differences in growth rate between individual colonies, but it does not necessarily follow that these differences are due to the treatments applied, though the probability that this is the case must be high, for all the colonies are derived from a single clone and grown under controlled conditions.

for the withdrawals of these samples and for the random removal of plants to prevent overcrowding when calculating the total number of fronds composing the colony. The experiment extended over eighteen days, but certain treatments were altered and others introduced upon the eleventh day.

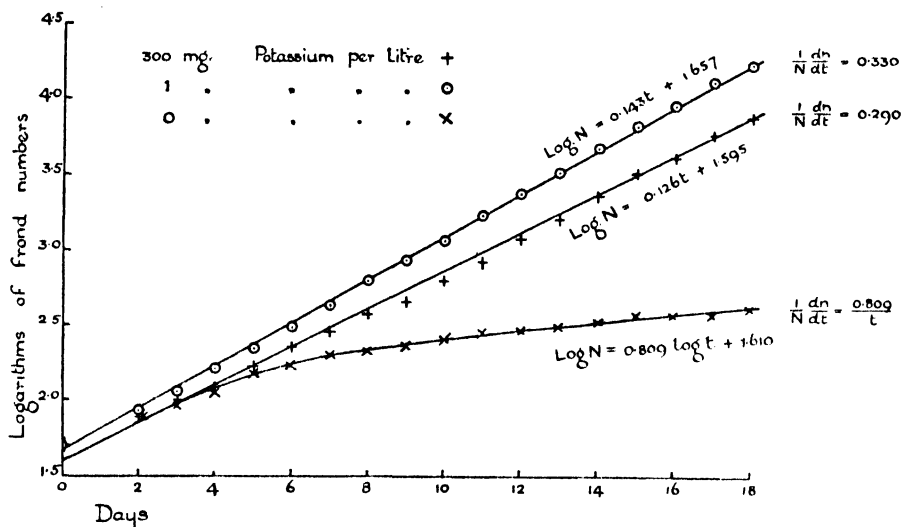


FIG. 1. Logarithms of frond number plotted against time.

The treatments, limited in number by the silica beakers available, comprised three during the first part of the experiment, each treatment being duplicated. These treatments were (a) no added potassium, (b) 1 mg. potassium per litre, and (c) 300 mg. per litre. The plants used for the duplicates had been under different environmental conditions and they must be considered as a parallel rather than a duplicate series. The rates of growth of one series are higher, at corresponding potassium concentrations, than those of the other. A statistical comparison between the mean rates by the methods introduced by Fisher (3) shows that such a group of differences would only have arisen by chance six times in a hundred. This suggests that environmental differences prior to the experiment may well have affected the rate of multiplication, despite the genetic similarity of the two sets of colonies, for all were members of the same clone, derived by vegetative reproduction from a single frond.

#### EXPERIMENTAL RESULTS.

*Frond number.* Fig. 1, in which the logarithms of frond number of the more rapidly growing series are plotted against time, shows that with 300 and 1 mg. potassium per litre the rate of multiplication is exponential, but with no potassium added the rate falls with time.

An essentially similar diagram may be drawn for the parallel series, but with lower rates of growth at each potassium concentration. By the method of least squares the following equations are fitted to the four exponential curves—

$$\begin{array}{lll} \text{1 mg. potassium per litre} & N = 45 e^{0.330t} \\ & N = 147 e^{0.289t} \\ 300 & \text{,,} & \text{,,} & N = 40 e^{0.290t} \\ & & & N = 143 e^{0.260t} \end{array}$$

*Curves of frond number of potassium-deficient colonies.* Fig. 2 shows that the curves of the logarithms of the frond numbers of the two colonies grown with no added potassium become linear when plotted against the logarithms of time.

Accordingly the relative growth may be represented by equations of the type  $\frac{1}{n} \frac{dn}{dt} = \frac{r}{t}$ , which on integration become  $n_1 = n_0 e^{r \log t} = n_0 t^r$ , where  $n$  is the frond number at any time  $t$  and  $r$  is a measure of the growth rate.

Curves of similar type were fitted by Gregory to the growth in area of the leaf surface of cucumbers at supra-optimal temperature (5) and with deficiency of light (4). The appearance of this relationship in such diverse circumstances suggests that it may well be of general application to biological growth that would have followed the compound interest law but for the presence of a deleterious or deficiency factor.

TABLE II.

*Frond Numbers of Colonies with no Added Potassium.*

Day.	Series I.			Series II.		
	Obs.	Calculation from equation A.	Calculation from equation B.	Obs.	Calculation from equation A.	Calculation from equation B.
0	150	—	150	50	—	50
2	268	257	267	80	71	80
3	324	346	335	91	94	93
4	446	428	409	113	125	118
5	508	504	485	150	149	139
6	572	575	562	174	173	161
7	623	646	638	206	197	184
8	708	711	712	221	219	207
9	751	775	782	236	241	230
10	857	838	848	264	262	253
11	922	897	909	290	284	277
12				302	304	298
13				314	324	320
14				339	344	341
15				381	364	361
16				381	384	380
17				382	403	398
18				419	422	415

Values are given in the third and sixth columns of Table II calculated from the following equations:

$$\text{Series I (A)} \quad \frac{1}{n} \cdot \frac{dn}{dt} = \frac{0.734}{t} : \log n = 0.734 \log t + 2.189.$$

$$\text{Series II (A)} \quad \frac{1}{n} \cdot \frac{dn}{dt} = \frac{0.809}{t} : \log n = 0.809 \log t + 1.610.$$

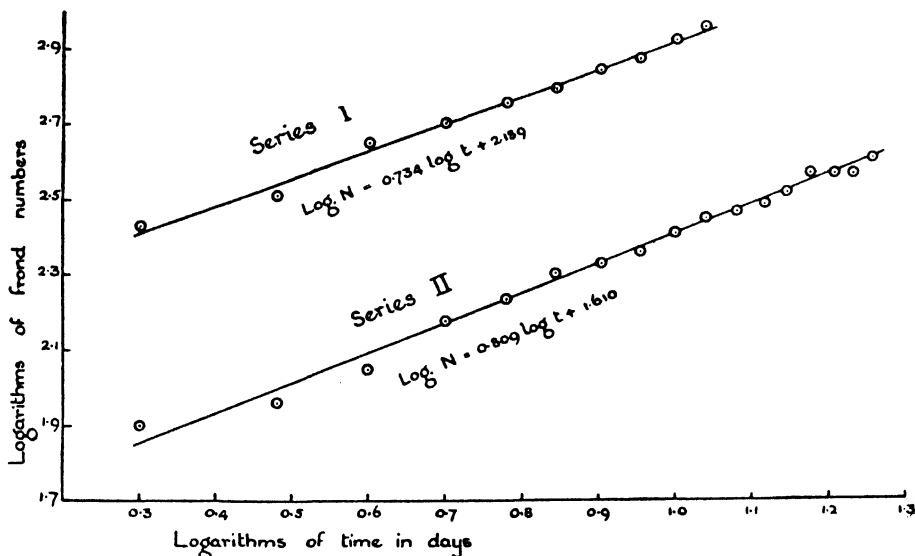


FIG. 2. Logarithms of frond number of potassium-starved colonies plotted against logarithms of time.

The calculated values in the table show good agreement with the experimental results from the second day onwards, but from the nature of the equation used the value of  $n$  becomes  $-\infty$  if  $t$  is reduced to zero. Moreover, it would appear advantageous to represent the growth rate of the Lemna colony as a decrement from the constant value maintained before deficiency conditions were imposed. The assumption made is that the relative growth rate falls exponentially with time, and therefore equations of the following type are used:  $\frac{1}{n} \cdot \frac{dn}{dt} = re^{-kt}$ .

The relative growth rate is given by  $\log \frac{n}{n_0} = \frac{r}{k} (1 - e^{-kt})$ , where  $n_0$  represents the initial frond number and  $n$  the frond number at any time  $t$ ,  $r$  represents the initial relative growth rate (multiplication rate) and  $k$  a constant, representing the daily percentage fall in relative growth rate from the initial value.

Values given in Table II (columns 4 and 7) are calculated from the equations given below.



$$\text{Series I (B)} \quad \frac{1}{n} \cdot \frac{dn}{dt} = 0.332e^{-0.149t} : \log N = 7.241 - 2.23e^{-149t}.$$

$$\text{Series II (B)} \quad \frac{1}{n} \cdot \frac{dn}{dt} = 0.262e^{-0.106t} : \log N = 6.38 - 2.47e^{-0.106t}.$$

The index 'r' in the exponential equation  $N_1 = N_0 e^{rt}$  denotes the constant rate of increase under conditions of full nutrient supply. The linear relationship between the logarithms of frond number and the logarithms of time (Fig. 2) shows that, under conditions of potassium starvation, *the rate of falling off from the exponential increase that would otherwise have been obtained remains constant.* The rate of increase during the period of starvation may be denoted either by the regression coefficient of the logarithms of frond number on the logarithms of time or, if it be assumed that the rate of fall is itself exponential, by a decrement from the rate at the beginning of the period. Thus the rate of increase of a colony (Series I) may be considered to be 33 per cent. compound interest per day and to fall with potassium starvation at a rate of 15 per cent. compound interest per day.

*Optimal potassium concentration.* After ten days' growth it was noted that the colonies with 300 mg. per litre were multiplying more slowly than those with 1 mg. per litre. Moreover, the rate of growth of the colony with no added potassium, that had been used for area and dry weight estimations, had become insufficient to replace the numbers of fronds removed for sampling. On the eleventh day one colony of each treatment was discarded (the slower growing series), sampling was carried on from the other (the more rapidly growing series) and intermediate treatments were set up at 20, 100, and 200 mg. per litre. The remaining colony with no added potassium was subdivided into daughter colonies, one being grown in a concentration of 1 mg. per litre and the other continued as before with no added potassium.

The daily exponential indices obtained for the relative rates of increase in frond number during this second part of the experiment are shown in Table III.

TABLE III.

*Relative Rate of Increase in Frond Number (with S.E.)*

1 mg. potassium per litre	0.329 ± 0.010
20   "       "       "	0.345 ± 0.023
100   "       "       "	0.336 ± 0.018
200   "       "       "	0.336 ± 0.018
300   "       "       "	0.312 ± 0.020

The optimal potassium concentration appears to be of the order of 20 mg. per litre but cannot be stated within narrow limits, for the fall in rate obtained by increasing the potassium concentration to 200 mg. per litre lies

within the errors of the experiment. A statistical comparison between the mean rates of growth of both series with 1 and 300 mg. concentrations yields the following probabilities:

	$\bar{x} - \bar{y}$ .	$t$ .	$n$ .	$P$ .
Series I.	0.289-0.260	1.04	16	0.32
II.	0.330-0.290	1.76	15	0.10

These estimates may be combined by dividing the sum of the mean differences by the standard error of the sum of the variances, giving  $t = 1.92$  for  $n = 31$  and  $P = 0.05$  (approx.). This suggests that 300 mg. per litre may be supra-optimal under this light intensity. It is of interest to note that the potassium concentration in Clark's solution is 312 mg. per litre.

*Area and dry weight.* The exponential curves of Fig. 1 show that the plant colonies, although under different environmental conditions prior to the experiment, assume rapidly a constant rate of frond-number increase. In contrast the average frond areas and dry weights continue to rise for fifteen days, suggesting the advisability in future experiments of allowing the colonies to become adjusted to the conditions before making observations. At the end of the experiment the cumulative differences between the colonies grown with 1 mg. potassium per litre and with no added potassium had risen to fifty times for area and thirty times for dry weight.

*Net assimilation rate.* The effect of potassium on net assimilation rate, estimated by  $\frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{A_2 - A_1}$  where  $A_1, A_2, W_1, W_2$  are the total area and total dry weight of the colonies at times  $t_1$  and  $t_2$  respectively, is shown in Table IV.

TABLE IV.

*Net Assimilation Rate.*

	Mg. dry weight per sq. cm. per two days.	Mg. $\text{Co}_2$ per sq. dcm. per hour. <sup>1</sup>
0 mg. potassium per litre	0.39	1.3
1 " " "	1.68	5.7
20 " " "	1.80	6.1
100 " " "	1.67	5.7
200 " " "	1.51	5.1
300 " " "	1.62	5.5

There is evidence of a lower net assimilation rate with no added potassium. This is confirmed by the net assimilation rate of the parallel series, which is 1.54 mg. dry weight per sq. cm. per two days for the colony in a solution with 1 mg. potassium per litre and 1.07 mg. dry weight per sq. cm. per two days for the colony with no added potassium.

<sup>1</sup> The assumption is made that the composition of dry weight corresponds to the formula for starch or cellulose.

*Average frond area and dry weight.* The dry weight per unit area of the colonies with no added potassium, 1 and 300 mg. per litre is given in Table V. The mean dry weight per unit area of the colonies with no added potassium is 52 per cent. and 58 per cent. higher than the colonies with 1 mg. per litre.

1 mg. potassium per litre .	2.24 ± 0.06	2.50 ± 0.04
no added potassium .	3.41 ± 0.24	3.96 ± 0.24

TABLE V.

*Frond Dry Weight (mg. per sq. cm.).*

Potassium (mg. per litre).	Series I.					
	300		1		0	
Day.						
0	—	3·16	—	3·16	—	3·16
2	3·46 3·53	3·50	2·98 3·10	3·04	— 3·03	3·03
4	2·27 2·08	2·18	2·17 2·24	2·20	3·60 3·60	3·60
6	2·46 —	2·46	2·26 2·42	2·39	2·90 2·96	2·93
8	2·59 2·84	2·71	2·03 2·16	2·09	3·22 3·74	3·48
10	3·54 3·08	3·31	2·32 2·26	2·29	2·78 3·06	2·92
11	— —	—	— —	—	4·06 4·34	4·20
Series II.						
						1 mg. potassium after eleven days with no added potassium.
11	2·58 2·41	2·49	2·64 2·53	2·58	3·92 3·88	3·90 — —
12	— —	—	— —	—	— —	3·16 3·16
13	— —	—	2·40 2·49	2·44	3·61 3·68	3·64 2·37 2·32 2·34
14	2·46 2·30	2·38	2·44 2·51	2·47	— —	2·24 1·94 2·09
15	2·35 2·51	2·43	2·36 2·27	2·31	4·24 4·32	4·28 2·22 1·19 2·20
16	2·50 2·68	2·59	2·54 2·57	2·55	— —	2·79 2·44 2·61
17	2·58 2·56	2·57	2·48 2·51	2·49	3·41 3·40	3·40 2·32 2·38 2·35
18	2·97 —	2·97	2·63 2·76	2·69	4·70 4·50	4·60 2·60 — 2·60

Area and weight data taken subsequent to the adjustment of the colonies to the experimental conditions are shown in Table VI.

TABLE VI.

*Average Frond Area (sq. cm.) and Dry Weight per Unit Area (mg. per sq. cm.).*

Potassium (mg. per litre).	Average frond area.				Mean.	Dry weight per unit area (sq. cm.).	
	Day 16.		Day 18.				
300	0.0420	0.0395	0.0350	0.0386	0.0390	2.50	2.59
	0.0370		0.0381			2.68	
			0.0427				
200	0.0484	0.0487	0.0449	0.0474	0.0480	2.38	2.25
	0.0491		0.0479			2.12	
			0.0494				
100	0.0434	0.0451	0.0424	0.0444	0.0447	2.49	2.48
	0.0468		0.0440			2.48	
			0.0469				
20	0.0381	0.0373	0.0357	0.0382	0.0377	2.63	2.62
	0.0366		0.0399			2.62	
			0.0389				
1	0.0425	0.0407	0.0396	0.0383	0.0395	2.54	2.55
	0.0390		0.0374			2.57	
			0.0378				
0	(Day 15)						
	0.0213		0.0211	0.0195	0.0228	4.24	3.84
	0.0222		0.0180			4.32	
		0.0244					
	(Day 17)						
	0.0258	—	—	—	—	3.41	
	0.0294					3.40	

Fig. 3 gives the average frond area plotted against potassium concentration. Table VI shows that the average frond area is significantly higher with 200 mg. potassium per litre than with 20 mg. The optimal potassium concentration for area is of a higher order than that for relative rate of increase in frond number and that for net assimilation rate, for these two rates show no significant increase in concentrations above 20 mg. per litre (Tables III and IV).

The high dry weight per unit area of the colony with no added potassium is clearly brought out in Fig. 4. The strong inverse proportionality between area and dry weight per unit area, throughout the range of potassium concentrations studied, corresponds with a correlation coefficient of  $-0.944$ . It may readily be seen that the average frond dry weight has been but slightly affected by variation in potassium supply.

The high dry weight per unit area of the colonies with no added potassium in conjunction with the low net assimilation rates of these colonies requires consideration. Some light is thrown upon this phenomenon by the behaviour of the sub-colony grown with 1 mg. per litre after eleven days with no added potassium, which is discussed below.

*Curves of recovery from potassium deficiency.* The average frond dry weight during recovery from potassium starvation together with the average

frond area is shown in Table VII and Fig. 5. On the addition of potassium the average frond dry weight falls, within a period of seventy-two hours, to 60 per cent. of its former value.

At the end of the experiment samples from each colony were killed

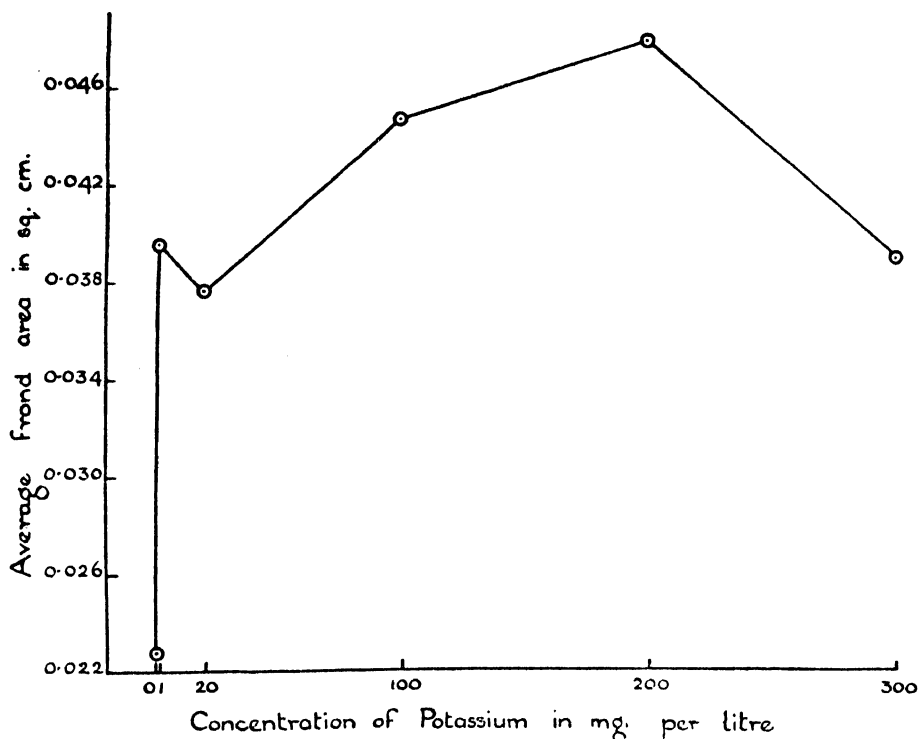


FIG. 3. Average frond area plotted against potassium concentration.

rapidly by heat, decolorized with alcohol, stained with iodine, and placed on a white plate. Whereas the fronds from the colony with 1 mg. per litre showed the presence of starch in moderate amount, those from the colony with no added potassium were completely black, demonstrating an abnormally high starch content. It seemed possible that the rapid fall in average frond dry weight of the potassium-starved plants, after being supplied with that element, was associated with the disappearance of starch accumulated during the period of potassium deficiency.

Fig 6 shows the logarithms of the frond numbers of the starved colony during recovery from potassium-starvation together with those of the colony given the same treatment since the beginning of the experiment. Recovery in relative rate of increase in frond number is complete after two days, and it is of interest to note that the exponential index of the colony that had been starved (0.318) does not differ from that of the colony grown

TABLE VII.  
*Frond Number, Frond Area, and Frond Dry Weight during Recovery from Potassium Starvation.*

Day.	Frond number.		Average frond dry weight (mg.).			Average frond area (sq. cm.).		
	1 mg. per litre.	No added potassium.	1 mg. per litre.	No added potassium.	1 mg. per litre recovery effect.	1 mg. per litre.	No added potassium.	1 mg. per litre recovery effect.
11	100	100	—	—	—	0.0376 0.0388	0.0382 0.0234	—
12	138	104	129	—	0.075	—	—	0.0237
13	195	108	158	0.100 0.088	0.094 0.079	0.0416 0.0353	0.0384 0.0220	0.0270 0.0311
14	282	117	228	0.098 0.097	0.098	0.0402 0.0386	0.0394	0.0268 0.0268
15	390	131	322	0.103 0.095	0.099	0.070 0.064	0.0428	0.0316 0.0292
16	538	131	435	0.108 0.100	0.104	0.096 0.083	0.0407	0.0344 0.0340
17	777	131	565	0.106 0.107	0.107	0.091 0.087	0.0417	0.0392 0.0366
18	1000	144	787	0.104 0.103	0.104	0.099 0.081	0.0383 0.0378	0.0211 0.0180

continuously with 1 mg. potassium per litre (0.329). Potentiality for subsequent rate of increase in frond number is retained unimpaired during a period of potassium starvation.

The rate of increase in area and the net assimilation rate of the starved

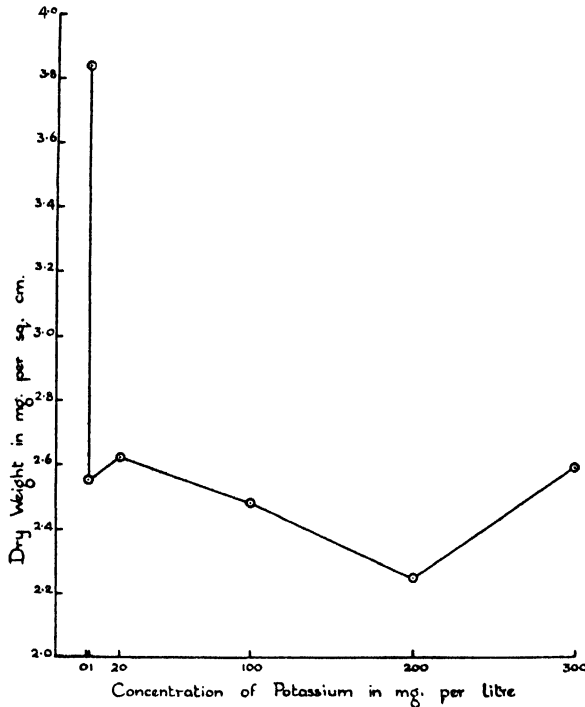
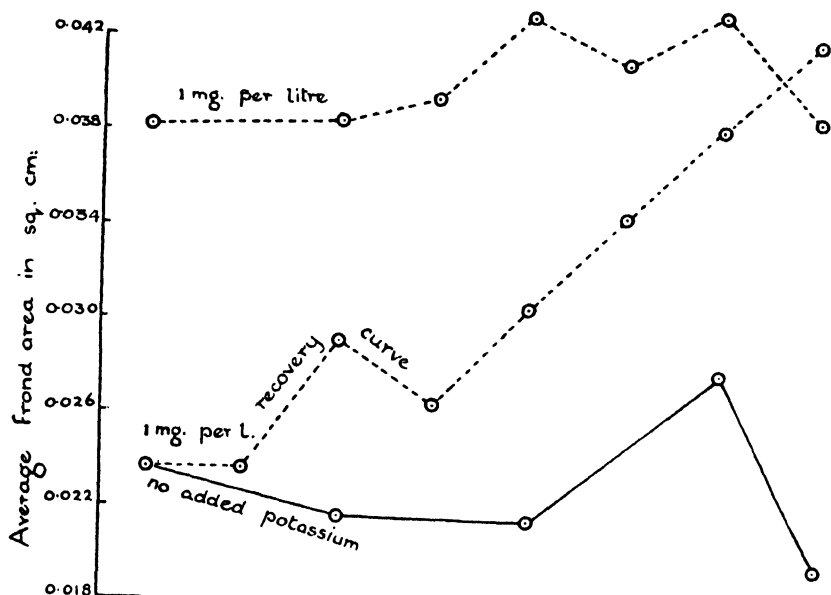


FIG. 4. Dry weight per unit area plotted against potassium concentration.

colony during recovery is shown in Fig. 7 in comparison with the mean of the colony grown continuously with 1 mg. potassium per litre, the curves being smoothed by averaging overlapping two-day periods. Fig. 7 also shows that the dry weight per unit area of the starved colony, forty-eight hours after transference to a solution with 1 mg. potassium per litre, falls to the level of the colony grown throughout with 1 mg. potassium per litre. Since the standard deviation of a single observation from the mean of the latter colony is 4.8 per cent. the additional fall of 15 per cent. is probably a real effect, indicating a transient phase of subnormality before final recovery.

Recovery in rate of increase in area is immediate, being complete in twenty-four hours, whereas recovery in net assimilation rate is much slower, requiring approximately seventy-two hours. After forty-eight hours the area of the colony has increased by 94 per cent., whereas the dry weight has

## FROND AREA



## FROND DRY WEIGHT

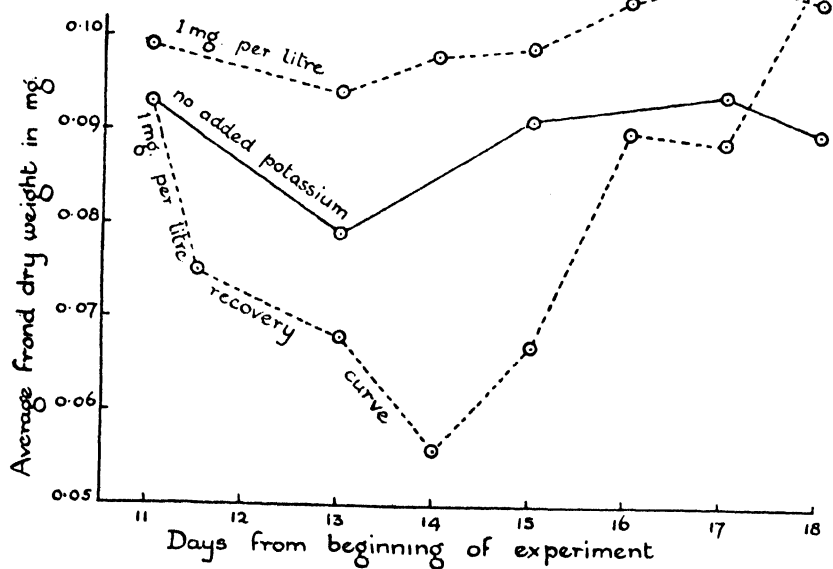


FIG. 5. Average frond area and dry weight of a colony previously potassium-starved plotted against days after transference (on the eleventh day of the experiment) to a solution with 1 mg. potassium per litre. The average frond area and dry weight of colonies (a) grown continuously in a solution with 1 mg. potassium per litre and (b) grown with no added potassium are also shown.



increased by only 16 per cent. (Table VII). The lag in recovery of net assimilation rate might be due either to failure of the mechanism controlling real assimilation rate to develop as rapidly as the new fronds which are formed increase in area, or to a temporary increase in respiration rate of

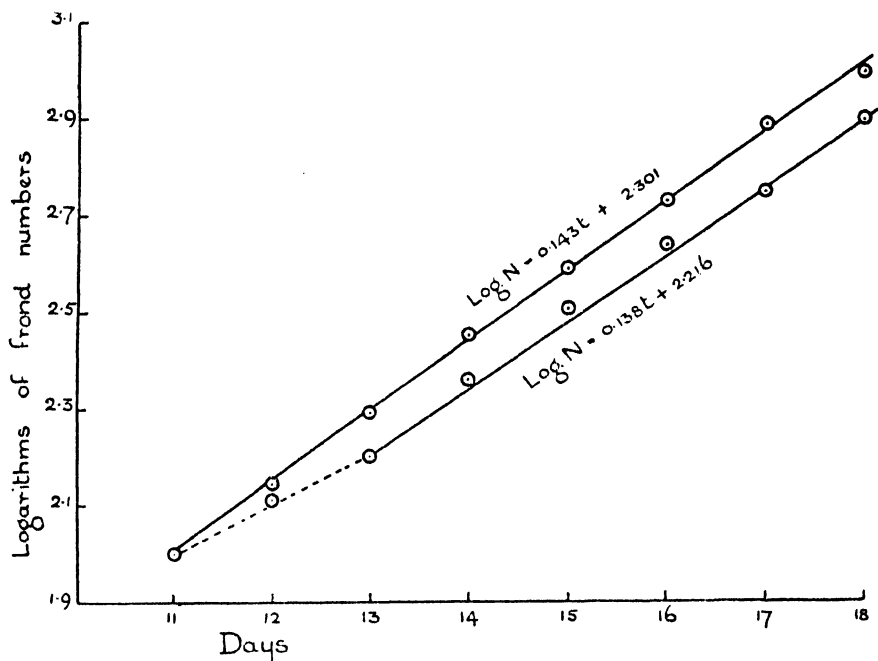


FIG. 6. Logarithms of frond number plotted against time of (a) a colony, previously potassium-starved, after transference (on the eleventh day of the experiment) to a solution with 1 mg. potassium per litre, and (b) a colony grown continuously in a solution with 1 mg. potassium per litre.

the old fronds, induced by an excess of surplus carbohydrate accumulated during starvation.

*Dry weight per unit area and starch content.* The rapid loss of dry weight on addition of potassium to a starved colony is scarcely compatible with the view that the dry weight per unit area is primarily a measure of the thickness of the frond, but it suggests that it is determined mainly by the starch content. An abnormally high starch content of the colony with no added potassium might arise if the relative rate of frond number increase falls so rapidly that, in spite of a low net assimilation rate, the carbohydrates accumulate for want of utilization. At the end of the first two days of the experiment, however, the colonies with no added potassium show no significant fall in relative rate of increase in frond number in comparison with the colonies grown with 1 mg. potassium per litre, whereas the dry weight per unit area already shows an increase beyond the limits of the sampling errors. On the fourth day from the beginning of the experiment the dry weight per unit area of a colony with no added potassium is already

64 per cent. higher than that of a colony with 1 mg. per litre, whereas the relative frond numbers only differ by 12 per cent. Moreover, the dry weight per unit area of the colonies with no added potassium does not rise with time, as might be expected if the high starch content was a process of

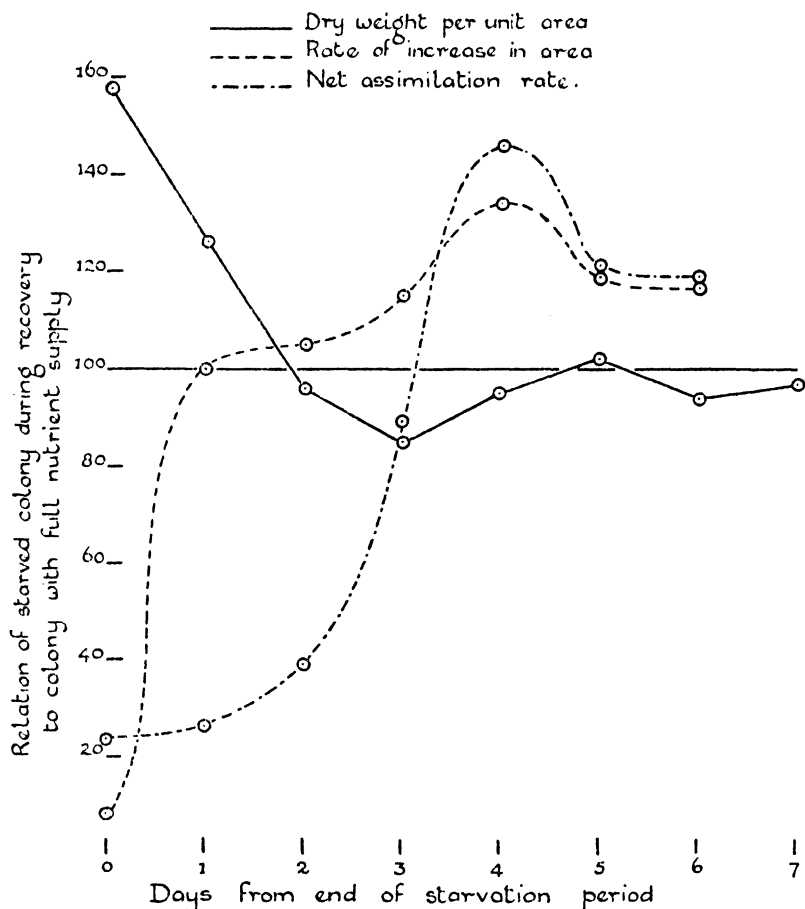


FIG. 7. Percentage relation in rate of increase in area and net assimilation rate of a colony transferred, after eleven days with no added potassium, to a solution with 1 mg. per litre, to the mean rates of a colony grown continuously in a solution with 1 mg. potassium per litre, which are represented by a straight line. The curves are smoothed by plotting the means of overlapping two-day periods. The daily percentage relation in dry weight per unit area is also shown.

gradual accumulation due to non-utilization. It remains relatively constant at a level of 3.41 mg. per sq. cm. for one series and 3.96 for the parallel series. Such phenomena do not support the view that the high starch content is a process of accumulation due to non-utilization, but they suggest strongly that the potassium level of the frond directly affects the starch content by control of the rate of starch hydrolysis.

*Effect of potassium on the rate of starch hydrolysis.* The working hypothesis suggested by these observations was tested in a later experiment. Four colonies were grown under the same lamp in solutions with concentrations of 200, 2.0, and  $\frac{1}{8}$  mg. potassium per litre and with no added potassium. The height of the lamp was adjusted to give a light intensity of 300 foot-candles.

After twenty days' growth the dry weight of samples from each colony was found and fresh samples taken calculated to be of approximately equivalent dry weight. These examples were treated as follows: (1) Corked in small tubes, after removing superficial moisture with blotting paper, until all the samples were ready. (2) Ground with approximately similar quantities of sand treated chemically so as to be potassium-free.<sup>1</sup> (3) The same volume of a starch solution added to each tube together with two drops of mercuric chloride. (4) Tubes incubated for twenty minutes at 40° C. to start the reaction. (5) Tubes, after the addition of the same volume of a dilute iodine solution to each, tightly corked and placed in the refrigerator at about 2° C.

The approximate times taken for complete hydrolysis of the starch were found to be those in Table VIII, fresh iodine being added to ensure that the disappearance of colour was not due to adsorption or volatilization of that previously used.

TABLE VIII.

*Hours taken for Hydrolysis of a given Starch Solution.*

200 mg. potassium per litre	$\left. \begin{array}{l} 3.5 \\ 4.5 \end{array} \right\}$	4.0 hours.
2 " " "	$\left. \begin{array}{l} 9.0 \\ 18.0 \end{array} \right\}$	13.5 "
$\frac{1}{8}$ " " "	$\left. \begin{array}{l} 18.0 \\ 28.0 \end{array} \right\}$	23.0 "
0 " " "	$\left. \begin{array}{l} 28.0 \\ 30.0 \end{array} \right\}$	29.0 "

Although the errors are large, there appears to be clear evidence of a retardation in rate of starch hydrolysis accentuated by decreasing potassium supply.

Criticism may be directed towards reference of the rates of starch hydrolysis to a weight basis. This was unavoidable, for it was not possible to find time to trace the areas with a planimeter until after the experiment was completed. The results are summarized in Table IX in terms of both area and dry weight, the assumption being made that the time taken for a colony to hydrolyse a given starch solution would be halved if the area of the colony were doubled.

<sup>1</sup> I am indebted to Dr. J. I. Armstrong for the provision of this sand.

TABLE IX.

	Relative frond dry weights.	Relative frond areas.	Relative times taken to convert given starch solution by a colony	
			(a) of equal weight.	(b) of equal area.
200 mg. potassium per litre	100	100	100	100
2 " " "	96	95	338	321
$\frac{1}{8}$ " " "	76	65	588	382
0 " " "	80	53	726	385

A colony of equivalent weight and area grown with no added potassium has taken 7.3 and 3.9 times as long respectively to hydrolyse a given quantity of starch as a colony grown with a sufficiency of potassium.

*Discussion of the relation of potassium to carbohydrate metabolism.* The effects of variation in potassium supply are summarized below: (1) Potassium-starved colonies are characterized by high dry weight per unit area (Table V) and high starch content (p. 184). On exposure to potassium starvation the dry weight per unit area rises *before* there is a significant fall in relative rate of frond multiplication (p. 182), and on transference of a starved colony to a solution with full nutrient supply the dry weight per unit area falls *before* recovery in relative rate of frond multiplication (Table VII). The rate of starch hydrolysis is retarded in potassium-starved fronds (Table VIII). Such phenomena suggest primarily a derangement of the starch-sugar balance, presumably by alteration of the conditions controlling amylolytic activity.

(2) Potassium-starved colonies, although characterized by high dry weight per unit area and high starch constant, have low net assimilation rates (Table IV). Should increase in respiration alone be responsible for these low net assimilation rates then, if the respiration rate of a colony with full nutrient supply is as high as 33 per cent. of the real assimilation rate,<sup>1</sup> the magnitude of the fall in net assimilation rate with potassium starvation would necessitate an increase in respiration rate of the order of over 200 per cent. An increase of this order seems improbable in view of the maintenance during starvation of a level of dry weight per unit area 50 per cent. higher than that of colonies with full nutrient supply. In the absence of direct respiration determinations the possibility that an increased rate of this process is responsible for part of the observed differences in net assimilation rate cannot be ruled out, but the weight of evidence favours the view that the low *net* assimilation rate of potassium-starved plants is due to a low rate of *real* assimilation. This low rate of real assimilation is presumably related to a progressive reduction of the normal frond colour, which was noted in the potassium-starved colonies.<sup>2</sup> The young fronds

<sup>1</sup> A value based on later experiments.

<sup>2</sup> The frond colour of a colony grown with 100 mg. potassium per litre approximated to bice

were deep green and appeared healthy, but yellowed rapidly on the approach of maturity.

(3) Variation in average frond area, induced by variation in potassium supply, is inversely proportional to variation in dry weight per unit area (Figs. 3 and 4). This inverse relationship is shown by the value of the 'correlation coefficient'  $r = -0.944$ . This effect holds for potassium-starved colonies with falling rates of multiplication and low net assimilation rates, and also for colonies grown with high-potassium levels, which do not affect multiplication and net assimilation. These observations seem compatible with the view that the average frond area is dependent on a starch-sugar balance, relatively less starch and higher sugar concentrations being associated with increased frond areas and lower levels of dry weight per unit area.

(4) The most striking feature of recovery from potassium starvation, following transference of a colony grown with no added potassium to a solution with 1 mg. per litre, is the immediate increase in frond area (Fig. 7) accompanied by a fall in starch content. The falling starch content is presumably associated with a high concentration of sugars, and it seems possible that it is the suction pressure exerted by these sugars that is partly responsible for the rapid increase in area rather than the intake of potassium corresponding to 1 mg. per litre in the external solution. This suggests that the low frond area recorded during starvation may have been associated with low sugar content. It cannot be determined whether all the sugars presumably hydrolysed during recovery from the starch accumulated during starvation are used in growth, or whether some are dissipated through high respiration. The lag in recovery in net assimilation rate might be due either to temporarily high respiration or to slow recovery to the normal photosynthetic level. It is probable that both effects are involved.

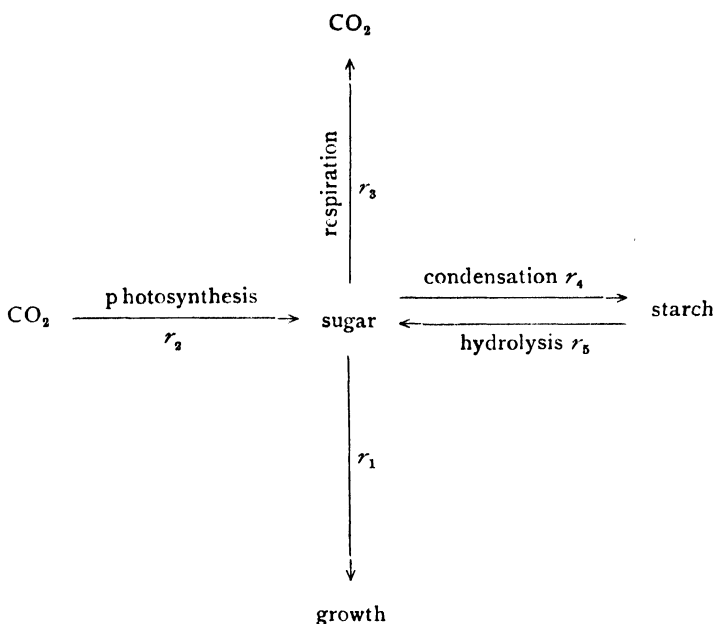
The considerations outlined so far support two contentions: (1) potassium affects the starch-sugar balance of the frond, starved fronds having a high starch but low sugar content. An effect of this kind is visible, not only in starved colonies, but also in colonies grown with high potassium levels that do not affect assimilation and multiplication. (2) Potassium affects the rate of real assimilation. The effect is restricted to the colonies grown with no added potassium in which the chlorophyll is notably reduced.

The starch-sugar relations in the frond may be diagrammatically represented as shown on the following page.

Sugar concentration is represented as regulated by the levels of five resistances in the paths of possible gain or loss of sugar. Sugar concentration is reduced by (1) loss of sugar used in growth ( $r_1$ ), (2) condensation to starch ( $r_4$ ), and (3) respiration ( $r_3$ ). Sugar concentration is increased by

green of Ridgeway's Colour Standards, and the frond colour of a colony grown with no added potassium to chrysolite green.

(4) photosynthesis ( $r_2$ ) and (5) hydrolysis of starch ( $r_5$ ). With full potassium supply an equilibrium between starch and sugar is established. Any changes in this starch-sugar balance due to potassium must arise



through the differential effects of variation in potassium supply on the resistance  $r_1 - r_5$ .

The relative rate of multiplication falls throughout the period of starvation. This fall would lead to reduced consumption of sugar in 'growth', which may be represented by an increase in the resistance  $r_1$ . Potassium starvation is characterized by low net assimilation rate, the experimental evidence favouring the view that the real assimilation rate is subnormal. This reduction in real assimilation rate corresponds to reduced sugar synthesis, which may be represented by increase in the resistance  $r_2$ . The net assimilation rate of a colony grown with no added potassium is 70 per cent. of that of a colony grown with 1 mg. per litre during the first part of the starvation period, and only 25 per cent. during the last part of the starvation period. This indicates that the falling multiplication rate during starvation is accompanied by a falling assimilation rate. The magnitude of the mean fall in multiplication rate is of the order of 55 per cent., and that in net assimilation rate of the order of 65 per cent. Decreasing consumption of sugars in 'growth' due to falling multiplication rate might tend therefore to be offset by decreasing synthesis of sugars due to falling assimilation rate. If the increase in resistance  $r_2$  is roughly counter-balanced by increase in resistance  $r_1$ , the net sugar concentration should

remain appreciably unchanged. It is clear that, under these circumstances, the starch-sugar balance might be determined by the remaining resistances so that *a high starch content and high dry weight per unit area is compatible in potassium-starved fronds with a low net assimilation rate.*

Data are lacking of the effect of potassium on respiration, represented by the resistance  $r_3$ . High respiration rate would lead to loss of sugar, which would be replenished by the starch reserves. Since these are not depleted, but tend to accumulate in potassium-starved fronds, a high respiration rate is not the major factor involved. Low respiration rate would be associated with decreased loss of sugar and tend to increased sugar content, whereas the experimental evidence suggests a low sugar content of potassium-starved fronds. These considerations do not rule out the possibility of respiration changes being associated with potassium starvation, but they indicate that any such changes are not *primarily* responsible for changes in the starch-sugar balance of potassium-starved fronds.

Experimental evidence for a differential effect of potassium starvation on the remaining resistances  $r_4$  and  $r_5$ , representing starch condensation and hydrolysis, is definite. Direct measurements of the rate of starch hydrolysis in potassium-starved fronds show that it is greatly retarded. Microchemical tests disclosing marked starch accumulation demonstrate that a corresponding slowing up of the opposite process of starch condensation is not taking place. On transference of a potassium-starved colony to a solution with full potassium supply the starch level is immediately reduced, and this effect antedates recovery in both net assimilation and multiplication rates. This effect is clearly associated with increased rate of starch hydrolysis, not accompanied by corresponding increase in rate of starch synthesis.

To account for these facts the following hypothesis is suggested. The concentration of sugar with which starch is in equilibrium varies with the level of potassium supply, the requisite sugar concentration necessary for the formation of starch being lower for low potassium supply. As the period of starvation proceeds multiplication leads to a reduction in the initial potassium content of the fronds so that a larger proportion of the carbohydrate synthesized is present as starch and a smaller proportion as sugar. Potassium-starved fronds are then characterized by rising starch and falling sugar content. Dry weight per unit area which measures starch and sugar concentration combined would not be appreciably affected, and this is shown experimentally to be the case. A falling real assimilation rate, if roughly balanced by a falling multiplication rate as in these experiments, would not necessarily affect these starch-sugar relations. With continuance of potassium starvation starch accumulation proceeds at the expense of sugar content and the dry weight per unit area tends to reflect mainly changes in starch content. On transference of a colony grown

with no added potassium to a solution with 1 mg. potassium per litre the accumulated starch is hydrolysed and utilized with such rapidity that within forty-eight hours the excess dry weight per unit area has disappeared, and the colony is multiplying at the rate normal for a colony grown with 1 mg. potassium per litre.

The considerations outlined in this discussion lead to the conclusion that a major role of potassium is to regulate carbohydrate metabolism through control of the starch-sugar balance by activation of amylolytic activity. There is also evidence of a low rate of photosynthesis of colonies exposed to potassium starvation.

#### SUMMARY.

1. A study has been made of the growth and reproduction of colonies of *Lemna minor* in a series of potassium concentrations ranging from no added potassium to 300 mg. per litre.

2. Under a continuous light intensity of 450 foot-candles at 25° C. the optimal potassium concentration for relative rate of increase of frond number and for net assimilation rate is of the order of 20 mg. per litre, but for relative rate of increase of area is 200 mg. per litre.

3. The rate of increase in frond number is exponential in all concentrations from 1 to 300 mg. per litre. The equation of growth may be

written  $N_1 = N_0 e^{rt}$  and the differential equation  $\frac{1}{n} \frac{dn}{dt} = r$ . With no added

potassium the rate of increase in frond number falls with time. The equation of growth may be written  $n_1 = n_0 t^r$ , and the differential equation

$\frac{1}{n} \frac{dn}{dt} = \frac{r}{t}$ . The rate of growth during potassium starvation may also be

considered as a decrement from the rate prior to deficiency, the change of rate during starvation following a logarithmic decrement law. The relative

growth rate may be represented by the equation  $\frac{1}{n} \frac{dn}{dt} = r e^{-kt}$ , and the

frond number at any time  $t$  by the equation  $\log n - \log n_0 = \frac{r}{k} (1 - e^{-kt})$ ,

$n_0$  being the initial frond number as experimentally determined.

4. Potassium-starved colonies are characterized by high starch content and high dry weight per unit area, but by low net assimilation rate. At the beginning of the period of starvation the dry weight per unit area rises rapidly to a relatively constant level; it falls rapidly on renewal of the potassium supply. Enzyme extracts of colonies which have been grown in potassium-deficient solutions show diminished capacity for hydrolysing starch.

5. Variation in average frond area induced by variation in potassium supply is inversely proportional to the corresponding variation in dry



weight per unit area. The average frond dry weight is but slightly affected by variation in potassium supply.

6. The relation of potassium to carbohydrate metabolism is discussed. The experimental results lead to the conclusion that a major role of potassium is to regulate carbohydrate metabolism through control of the starch-sugar balance by activation of amylolytic activity. There is also evidence of a low rate of carbon assimilation of colonies exposed to potassium starvation.

Grateful acknowledgements are due to Professor V. H. Blackman for facilities for carrying out the work, and to Dr. F. G. Gregory and Dr. E. J. Maskell for assistance in the preparation of the paper.

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# The Effect of Pigment on Phototropic Response: A Comparative Study of Reactions to Monochromatic Light.

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With Plate III and six Figures in the Text.

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## I. INTRODUCTION AND PREVIOUS WORK.

MUCH work has been done on the response of organisms to light ; naturally, all of the earlier and a large part of the later work being qualitative in nature. The first attempt to express the photic sensibility of plants in quantitative terms was made by Wiesner (27) working on *Vicia*. Since the publication of his ' *Die heliotropischen Erscheinungen im*

[*Annals of Botany*, Vol. L. No. CXCVIII. April 1936.]

Pflanzenreiche', little advance was made along quantitative lines until 1909, when Blaauw (2) published 'Die Perzeption des Lichtes'. In this important contribution modern physical methods were for the first time employed, and its complete disagreement with all previous results led to the earlier work being discredited on the score of faulty technique.

More accurate work still was done by Parr (22). Working in the visible region of the spectrum, she found with *Pilobolus* a steady increase in the efficiency of the wave-lengths from the red to the violet end. Castle (4) repeated the work of Blaauw but on a different species of *Phycomyces*, and obtained a similar result: maximum response occurred in the violet region, and a falling off both in ultra-violet and the longer wave-lengths, so that when graphed something like an absorption spectrum was produced (see Text-fig. 4). He suggested that the difference between his results and Blaauw's might be due to the pigment content of the sporangioophores.

Johnston, Brackett, and Hoover (13), working on *Avena*, found the blue Wratten filter most efficient of the three colours, blue, green, and yellow. Blue was 30 times as efficient as green, and green 1,000 times as the red-yellow. The response of *Avena* had also been investigated by Sonne (23), who found that white light was 400 times as efficient as green, and 600 times as yellow, but that blue light needed only 3 per cent. of the energy of white light to cause the same minimal intensity response. Violet light was about half as potent as blue, while in ultra-violet a steady and slow decrease in efficiency became apparent (see Text-fig. 3).

Meanwhile Mast (19) had concluded a whole series of kindred investigations (Gross 9, Holmes 11, Jennings 12, Laurens 15, Yerkes 28) on the phototactic responses of motile algae to monochromatic light, by discovering that the difference in response was due to the extent of penetration of the light-sensitive zone (photo-receptor of *Volvox*, or pigment spot) by the various wave-lengths. Again, the blue-violet region was most efficient, the ultra-violet and the red ends of the spectrum showing a decrease in potency.

## II. STATEMENT OF PROBLEM.

When the relative efficiencies of the various parts of the spectrum were graphed (see Text-fig. 4) in the way suggested by Castle (4), something very much resembling an absorption spectrum having a maximum in the blue-violet region was given. This might conceivably be traced to some light-sensitive substance which acted as growth-regulator. But the slightly divergent results of all recent workers, and the insistence upon the presence of two maxima by all who had worked on green plants (e.g. Guillemin 10, on cress and mustard, Wiesner 27, on *Vicia*, and Dandeno 7, on various

green seedlings), made this appear doubtful. The conclusion of Mast (16) seemed to point rather to the light-gradient hypothesis of Nuernbergk (21), and the divergent results might be due to differences in the light-gradient across the sensitive regions of the plants in question. One of the reasons, however, for the large volume of conflicting data in the past was that each worker used different materials and different methods.

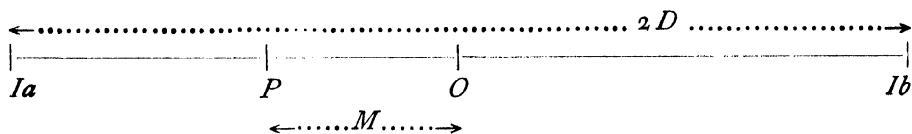
The present investigation aimed at a final settlement or possible reconciliation of these antagonistic points of view by a comparative study of a wide range of plants. The procedure employed was to assemble an apparatus which would give a high degree of accuracy, and, keeping the experimental conditions constant, ascertain the relative potency of response in the different parts of the spectrum for seedlings of many and varied types. The choice of plants for investigation was determined at first by previous work in this direction, but later by reasons which emerged in the course of the experiments. As the same apparatus was used in constant conditions, any differences in response could be attributed to some factor present in the plant under consideration. It was hoped to correlate such differences either with the photosensitive substance hypothesis, or with the light-gradient hypothesis, and, in view of the latter, detailed histological examination of the tips of seedlings responding was carried out.

### III. METHOD AND MATERIALS.

The method used by Blaauw (2), Parr (22), and Sonne (23), was to take a series of coloured lights of known intensity, at standard distance, and measure the minimum presentation time needed to obtain any curvature whatever. This seemed unsatisfactory, as the tonus of the plant varies even if conditions are kept constant. By taking a large number of cases, however, a fairly reliable average could be arrived at. Du Buy and Nuernbergk (8), and Bergann (1) have since used this method.

A better method, and one which was considerably quicker (an important factor in a large comparative study of plants), and which avoided the effect of several variable factors, was that used by Castle (4), by Johnston, Brackett, and Hoover (13), and by Mast (16) in his phototactic work, viz. the exposure of the plant simultaneously to lights from opposing directions. Thus if a plant is placed midway between two differently coloured lights (i.e. two sources of monochromatic light of different wave-lengths) of equal intensity (i.e. energy value), the curvature towards one in preference to the other would show a greater efficiency in inducing curvature in the wave-lengths chosen. This relative efficiency could be expressed in quantitative terms if the plant were moved towards the less efficient light

until a point was reached where the effect of one light was exactly counter-balanced by the other and the plant remained vertical. The position of this point (*P*) with respect to the distance from each light source would give a measure of relative efficiency according to the equation:



$$\frac{y}{x} = \left( \frac{D+M}{D-M} \right) \cdot \frac{Ia}{Ib}$$

*Ia* = Energy value of First Light.

*Ib* = Energy value of Second Light.

*D* = Half Distance between Light Sources.

*M* = Distance between Vertical Point and Midpoint.

*x* = Efficiency of First Light.

*y* = Efficiency of Second Light.

In practice it was found unnecessary to have the opposing light sources of equal intensity, so long as their relative energy values could be determined. By keeping the two light sources a considerable distance apart, (about 120 cm.), a fairly high degree of accuracy could be obtained. The probable error is estimated at less than  $\pm 0.5$  per cent.

Mast (16) used an interesting variation of this method, which was necessary since he was dealing with organisms (Flagellates) which are not radially symmetrical to the incident lights. His light sources were at right angles, and the divergence from the median in angle of direction of movement gave a measure of the relative efficiency of the wave-lengths employed. In certain cases, the tips of plants are not radially symmetrical, a cross section of *Avena* being oval in shape, but this is remedied by placing the seedlings with the long axis of the transverse section in the same direction as the incident beam of light. So long as the light sources are directly opposite, the irregularity of structure can be ignored.

A further refinement in the method was to use a tray of about 20 seedlings placed so that each one was fully exposed to light from both ends. By plotting as a graph the angles of curvature of all of these and determining the vertical point from the graph, the margin of error due to slight individual variation was greatly reduced. The method of Johnston, Brackett, and Hoover (13) of using a single seedling and moving the lights backward or forward until the effects balanced, gave only an approximate result with as much as  $\pm 5$  per cent. probable error. The method of using a large number of seedlings saved the unnecessary repetition of experiments to find the precise vertical point, and also afforded a means of detecting at

once such an occurrence as negative bending due to heat or excessive light, to which many of the divergent results of earlier workers are probably due. When exposed between two opposite light sources, a negative bending away from one might easily be mistaken for a positive bending towards the other, but when a number of seedlings are used, the angle of curvature will be greatest in the one nearest the light which is producing the effect, and least in the one furthest from it. Thus negative and positive bendings cannot be confused.

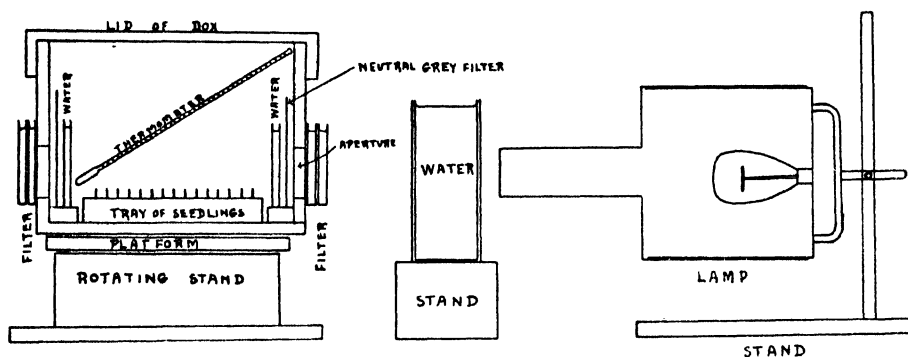
The graph of a whole series of curvatures should also reveal the effect of regions of the spectrum which are generally regarded as possessing no power of curvature (e.g. red), since, while not approaching the threshold of stimulation, these wave-lengths could counteract to a slight extent the colours lower down the spectrum. When, for instance, a tray of seedlings was exposed to yellow light for four hours, and the resulting angles of curvature graphed (angle against distance from light), a curve resulted which was a little steeper than that expected according to the inverse square law. (The departure from the inverse square law is probably due to the effect of geotropism). When the yellow light was opposed to red, and seedlings placed between them for four hours, the curve steepened considerably, the angle of those seedlings at the red end being reduced by about one-quarter, while that at the yellow end was reduced by about one-tenth. This result agrees with the response of *Avena* to high intensity of red light (claimed by Zollikofer 29, Bachmann and Bergann 1), and also explains the anomaly that red light has been shown to check the growth of *Avena* and yet does not induce curvature. Evidently red light produces a tropic effect on the *Avena* coleoptile, but it is below the threshold of stimulation for moderate intensities of light.

*Apparatus:* All the experiments were conducted in a dark room with double doors, the interior being coated with dull black so that no light was reflected from the walls or ceiling. As this room was in the centre of the building, the conditions remained practically constant, and thermometer readings taken at regular periods showed little variation. The important constituents of the apparatus were:

(a) *Standard source of light:* If seedlings were to be grown between opposing lights, it was imperative that standard lamps should be used and a constant current supplied to them. Parr (22) used storage batteries; but a less troublesome and more exact method was to use one lamp on the ordinary A.C. circuit, and make the tray of seedlings rotate so that they were illuminated through apertures at each end alternately at half-minute intervals. Variations in light intensity were thus balanced over the period of the experiment, and the cumulative effect of the flicks of light was equivalent in effect to constant exposure between opposing lights (Nathansohn and Pringsheim 20). The revolving mechanism was run on the same

electric supply, but variations in speed could be discounted since the fraction of total illumination received by the plants was constant without reference to the speed of rotation. Centrifugal force was found to be so small that it could be ignored.

As a source of light the Mazda gas-filled 'Daylight' globe (150 watts) was found most suitable, the one globe being used throughout the series



TEXT-FIG. 1. Apparatus used in phototropic experiments.

of experiments. The filaments of the globe were small and were arranged in a plane transversely to the length of the globe, so that distances from the source of light could be measured very exactly. The blue glass used counteracted to some extent the undue proportion of yellow and red in the light emitted.

The light was enclosed in a box of tin foil blackened on the outside, and with a cylindrical funnel nine inches long at the front. This threw a parallel beam of three inches diameter. After leaving the lamp, the rays of light passed through a glass vessel containing a six-inch depth of 0.01 per cent. copper sulphate solution in water. This cut out all wave-lengths above  $8000 \text{ \AA}^\circ$ . The glass vessel was painted black except for an aperture on each side, and was covered with a blackened screen to prevent diffraction upwards.

(b) *Light filters*: Parr (22) had used a spectrum projected through a narrow slit so that a band of light  $100 \text{ \AA}^\circ$  wide was allowed to fall upon the solitary plant being tested. This could not be applied to my apparatus. The use of Wratten colour-filters was at first contemplated (following the method of Johnston, Brackett, and Hoover (13)), but coloured solutions were found preferable. In all cases these were made of distilled water, and were contained in special cells of colourless glass. The wave-length of the light which these solutions passed was measured with a Hartridge spectrometer (Table I) in front of the 150 watt daylight lamp. All were

stable to light for at least the period of exposure in the experiment. The composition of the filters is given in Table I.

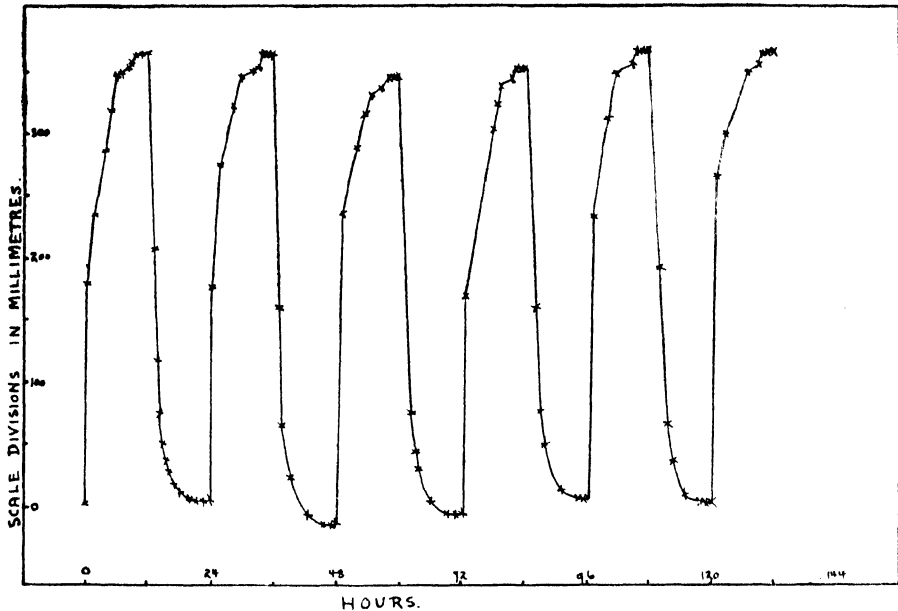
TABLE I.  
*Light Filters.*

Colour.	Range of wave-lengths.	Maximum.	Composition.	Thickness.
Red.	6170-7480A°	6500A°	Safranin 1 gm. in distilled water 950 c.c.	1.6 cms.
Red A.	6780-7390	7060	Safranin 1 gm. in distilled water 720 c.c.	0.8
			Aniline green 1 gm. in distilled water 8,500 c.c.	0.8
Red B.	6198-6730	6450	Safranin 1 gm. in distilled water 720 c.c.	0.8
			Naphthol green 1 gm. in distilled water 950 c.c.	0.8
Yellow.	5598-5790	5700	Eosin 0.5 gm. naphthol yellow 0.25 gm. in 1,000 c.c. distilled water.	0.8
			Naphthol green 2.5 gm., naphthol yellow 0.15 gm. in 1,000 c.c. distilled water.	0.8
Green A.	5250-5580	5450	Naphthol green 3 gm., toluene blue 0.25 gm. in 1,000 c.c. distilled water.	1.6
Green B.	4950-5220	5100	CuSO <sub>4</sub> 2 gm., distilled water 600 c.c., 40% ammonia 200 c.c. Add 400 c.c. 0.5% pot. dichromate.	1.6
Blue-green.	4480-5290	4860	CuSO <sub>4</sub> 2 gm., distilled water 900 c.c. 40% ammonia 300 c.c. Add 30 c.c. 0.5% pot. dichromate.	1.6
Blue.	4570-4940	4640	CuSO <sub>4</sub> 2 gm., distilled water 500 c.c. 40% ammonia 200 c.c. Add 10 c.c. 0.6% pot. dichromate.	1.6
Blue-violet.	3930-4790	4480	CuSO <sub>4</sub> 5 gm., 15% ammonia 1,000 c.c.	1.6
Violet.	3880-4430	4220	CuSO <sub>4</sub> 10 gm., toluene blue 1 gm. in 15% ammonia 1,000 c.c.	1.6
Ultra-violet.	3000-4230	3580	Specially prepared quartz glass filter with CuSO <sub>4</sub> 1 gm. in distilled water. 1,000 c.c. in quartz glass cell.	0.8

(c) *Measurement of light intensity.* As the A.C. supplying the lamp came from the ordinary electric light main and consequently fluctuated slightly, it was found advisable to use a thermopile with sufficient brass-work to give a reservoir of residual heat, and to arrange a standard time of



day for taking readings. A Nobili-Gallenkamp (London) thermopile of 60 thermocouples arranged in a circle, was placed at a standard distance behind the light filter and lamp, with one water-cell between the lamp and filter and another between the filter and thermopile. It was coupled with a Cambridge A.M. galvanometer, and the reading taken in scale-division



TEXT-FIG. 2. Measurement of blue-violet light by thermopile.

units. Light was allowed to fall upon the thermopile for 12 hours (8.30 a.m. to 8.30 p.m.) and readings taken at definite intervals. Then a period of 12 hours was allowed for the instrument to return to zero. An average of six such readings was regarded as sufficient, Saturday and Sunday being always omitted. Though a cumbersome and laborious method, remarkable uniformity of results was obtained both in the six readings, and in subsequent tests of the same filters.

Text-fig. 2 shows a graph of six readings obtained in measuring blue-violet light. Though both the zero and final readings fluctuated, the difference between them was fairly constant. It was in the six cases: 361.4, 359.9, 360.2, 359.8, 360.8, 361.1. Average 360.5. The upper part of each curve in the daily graph, when plotted in more detail, bears a close similarity to the amount of current supplied at the same period in the municipality. The rise and fall of the zero readings also corresponded to slight changes in the temperature of the dark room.

(d) *The method of measuring curvature.* Prior to each experiment the seedlings were arranged in the tray and left for four hours in darkness.

They were then finally tested with plumb-bob and horizontal microscope to see that the tips were quite vertical. This was done while exposed to illumination which did not affect curvature. At the close of the experiment the tray was immediately photographed, the subsequent distances of the seedlings apart and measurements of curvature being done at leisure on the photographs. Discrepancies due to parallax were so slight as to be negligible.

#### IV. EXPERIMENTAL DATA AND RESULTS.

(a) *Investigation of relative efficiency of different wave-lengths in promoting curvature:* *Avena sativa*. It was decided that *Avena sativa* should be the first plant tested, so that the result could be compared with those of Sonne (23), and Johnston, Brackett, and Hoover (13) (see Text-fig. 3). Substantial agreement would prove the apparatus satisfactory, and the comparative work could be proceeded with.

*Avena* was first submitted to a long series of preliminary tests, most of which merely confirmed the results of earlier workers. When trays of coleoptiles were exposed before each of the light filters separately, a rough indication of the subsequent tests was given. No bending occurred under the influence of either infra-red or red light, but a positive bending in increasing degree as the wave-lengths became shorter, until with violet and ultra-violet it slightly decreased once more.

The oat coleoptiles were then submitted to pairs of exactly similar filters, and careful experiments carried out to find whether the vertical point in these cases did coincide with the midpoint, as was expected. One of the photographs of this series is given in Pl. III, Fig. 1. In twelve experiments of this type the error in no case amounted to more than 0.15 cm., which as the light sources were 120 cm. apart gave a high degree of accuracy.

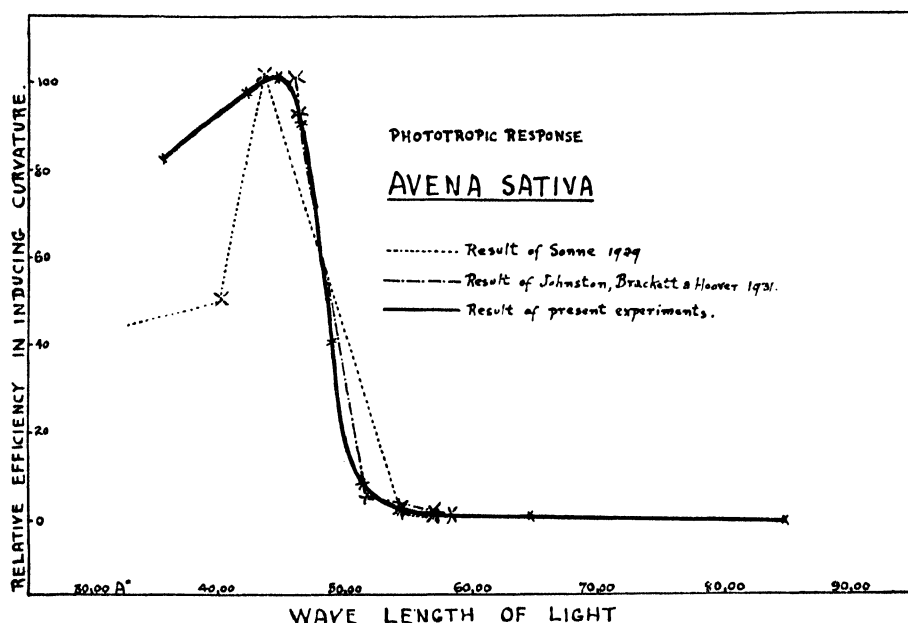
Pairs of different coloured filters were then used, and two experiments carried out with each pair. The results were so close that more than two was considered unnecessary. The following Table, which shows the ratios between the different groups of wave-lengths used, also shows how a cross-checking was carried out between the various filters to ensure accuracy.

The ratios only give the relative values between the various light filters, but by giving an arbitrary value of 100 to the most efficient filter, in this case blue-violet, the values of the others can be calculated proportionately.

This admits of a graph being drawn showing the relative efficiency of response for the different regions of the spectrum.

Castle (4), who devised this method of graphing the results, imagined that he was thus obtaining the absorption of spectrum of some substance affected chemically by light. Text-fig. 3 shows the result for *Avena* in

comparison with those of Sonne (23), and Johnston, Brackett, and Hoover (13) and Text-fig. 4 compares it with the work on *Pilobolus* (22) and *Phycomyces* (24).



TEXT-FIG. 3. Comparison of results for *Avena*.

TABLE II.

	Pairs of light filters.		Ratio of efficiency.
i	Violet	and ultra-violet	1.173 : 1
ii	Blue-violet	„ violet	1.043 : 1
iii	Violet	„ blue	1.086 : 1
iv	Blue	„ ultra-violet	1.082 : 1
v	Blue-violet	„ blue-green	2.252 : 1
vi	Blue-green	„ green B	6.049 : 1
vii	Blue-violet	„ green B	13.65 : 1
viii	Green B	„ green A	3.414 : 1
ix	Blue	„ green A	41.06 : 1
x	Green A	„ yellow	107.5 : 1
xi	Yellow	„ red	0 : 1
xii	Green B	„ yellow	365.0 : 1
xiii	Violet	„ green B	13.05 : 1
xiv	Blue-violet	„ green A	46.25 : 1
xv	Blue-violet	„ white	4.202 : 1

All show a maximum effect in the blue-violet region of the spectrum, with a falling-off both towards the red and the ultra-violet.

In this respect *Avena* can be taken as typical of colourless plants, since *Zea mays*, *Triticum*, and various species of *Panicum* which were tested, gave a closely similar result.

(b) *Lepidium sativum*: The hypocotyls of various green plants were now investigated, and cress, grown in sunlight until the hypocotyl had straightened, was found most suitable in size and sensitivity. Preliminary

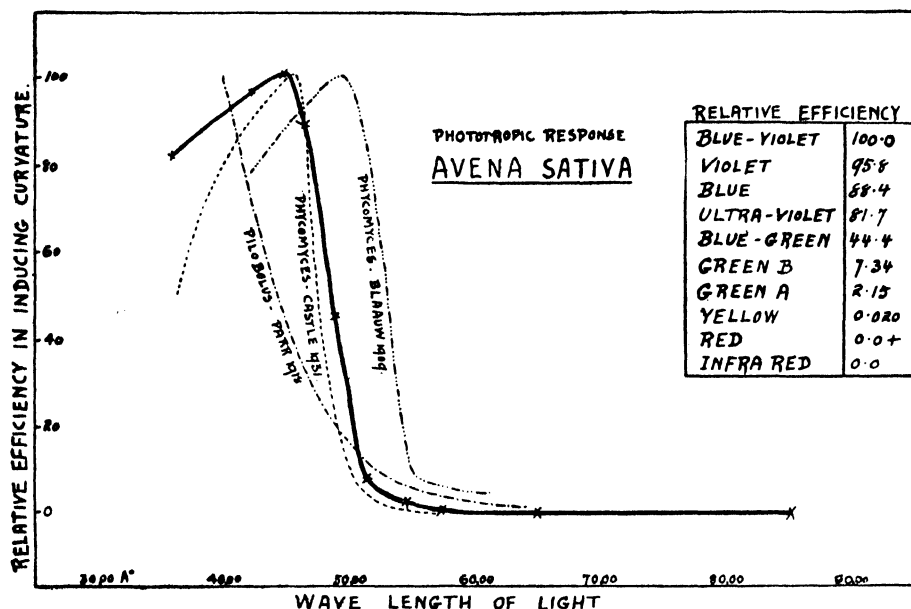


TABLE III.

*Relative Efficiency of Filters. Avena.*

Maximum wave-length.	Colour.	Efficiency.
4480 Å°	Blue-violet	100.0
4220	Violet	95.8
4640	Blue	88.4
3580	Ultra-violet	81.7
4860	Blue-green	44.4
5600	White	23.8
5100	Green B	7.34
5450	Green A	2.15
5700	Yellow	0.02
6500	Red	0.0 +
8500	Infra-red	0.0

tests were made in which Raphanus, Helianthus, Vicia, Pisum, and Brassica were exposed to the same filters as *Lepidium*, and even exposed simultaneously by mixing the seedlings in the tray and thus ensuring similar conditions of temperature and humidity, and the qualitative response was identical. *Lepidium* was therefore taken as typical of green plants in general.

When exposed to the series of filters, the green plant showed at first a complete absence of response in the green region of the spectrum, and also in the infra-red, but a definite response in the red (see Pl. III, Fig. 2) and of course in the blue-violet. This agrees with the results of Guillemin (10) working with cress and mustard, with Wiesner (27) on *Vicia*, and Dandeno (7) on various green seedlings. To make sure that conditions had not altered in any way since the *Avena* experiments were carried out, cress and oats were set alternately in the one tray and exposed between red and green light. In the photograph (see Pl. III, Fig. 3) the oats can be seen curving towards the green, while the cress goes to the red. It appears that the difference in response of the two plants must be accepted.

Now the *Lepidium* hypocotyls used had been grown in sunlight, while the *Avena* coleoptiles were etiolated. A series of experiments was now carried out to determine how far the difference in response might be due to the chlorophyll content.

(i) Cress was germinated and grown while exposed to green light in the apparatus. It was thus etiolated. The hypocotyls did not straighten but curved towards the green light from the beginning, though even now the effect was so slight that it could not be used to give any measurable efficiency to green light in the later experiments.

(ii) Cress was germinated in darkness, and grown until the hypocotyls had straightened. It was then exposed to green light and showed a slight curvature. In both these experiments the curvature was probably due to the much greater sensitivity of the etiolated coleoptiles, which was displayed also in the presence of other regions of the spectrum.

(iii) Etiolated cress was exposed to red light, and as chlorophyll would rapidly form at normal temperatures, ice was placed within the apparatus to keep the temperature low. At the end of 48 hours no curvature had taken place. Examination showed that a little chlorophyll had formed in the cotyledons, but none in the hypocotyl or growing tip.

As the growth-rate had been considerably reduced by the low temperature, no conclusion could be drawn until the experiment had been repeated under identical conditions, but with much weakened violet light instead of red. The violet light was weakened with neutral filters until its efficiency approximated to that of red for normal green seedlings. The hypocotyls now curved within 4 hours. To make quite sure, identical hypocotyls were now exposed to red light for 48 hours and to violet for 4 hours with the same result.

Cress seeds were now germinated inside the apparatus under a constant exposure to red light, but with ice present to hinder the formation of chlorophyll. The hypocotyls straightened vertically and showed no curvature, though they immediately responded to weakened violet light.

It was concluded that response of *Lepidium* to red light was due to the presence of chlorophyll.

(iv) The behaviour of *Avena* was now further studied to see whether it could be induced to yield responses similar to the green *Lepidium*. As a preliminary, the phototropic life-history of the oat was carefully investigated.

The coleoptile encloses the first leaf which develops within it, and finally pierces the tip. The coleoptile then forms a sheath round the base of the stalk and withers away. When the tip is pierced the coleoptile loses its sensitivity to light. The time taken for the first leaf to emerge varies considerably according to temperature, illumination, &c.: in daylight it occurs about 6 hours after the first appearance of the coleoptile from the glume, while in darkness it can be delayed for about 40 hours. If grown in sunlight, the coleoptile has a slight green appearance, but contains very little, if any, chlorophyll. The green appearance is due to the first leaf, and the colour deepens as the leaf develops. Above the level to which the first leaf has attained, the coleoptile tip appears almost transparent. In maize, on the contrary, the coleoptile contains quite an appreciable amount of chlorophyll, if grown in sunlight.

When the coleoptile tip has been pierced, the power of curvature to light is lost, but it disappears only gradually. A slight and continually decreasing response persists for about half an hour after the first leaf has emerged. Then follows a period when the oat plant is quite insensitive to light, normally of short duration, but capable of being lengthened considerably by etiolation. Exposure to the strongest illumination for 12 hours failed to effect curvature at this stage.

The second leaf then emerges from within the first, arising from the first node of the stalk, and so on with further leaves. The stalk elongates by means of a growing zone beneath the node, and the leaves are lifted above the sheathing coleoptile and finally separated from each other. This growing zone at the node is sensitive to light, and once again *Avena* shows tropic curvatures.

The first series of experiments was designed to test the bending at the node in the second leaf stage. Seedlings were grown in sunlight and submitted to the series of light filters. No bending occurred in green light after an exposure of 48 hours, but a slight yet definite curvature to red occurred after 30 hours (see Pl. III, Fig. 4). The response to the other filters was sluggish but normal. Etiolated nodes showed no response to red light, but a normal response to green. They were very unwieldy and difficult to handle at the second leaf stage. Evidently, the presence of chlorophyll in the light-sensitive region of *Avena* makes it susceptible to red light also, while it loses the response to green light.

(v) Another series of experiments was carried out on the *Avena* coleoptile itself, taking advantage of the short interval of reduced sensitivity during the emergence of the first leaf through the tip. A phototropic

curvature can be straightened out by removing the stimulus within a short time after exposure, so long as the plant is plastic enough to regain its vertical position. The normal curvature starts immediately below the tip, and then is transferred to the regions lower down the coleoptile successively, while the tip itself straightens. A small stimulus may then be obliterated by the geotropic stimulus which follows upon the removal of the unilateral illumination unless there has been time for permanent growth effects to ensue.

It was found that oat coleoptiles, grown in sunlight and transferred to the apparatus just as the first leaf was commencing to pierce the tip, would bend slightly to green light and then straighten out, whereas etiolated coleoptiles at the same stage would remain permanently bent. Coleoptiles grown under vertical red illumination so that chlorophyll had developed in the first leaf, also bent slightly to unilateral green light, and then regained their erect posture. It was not entirely a question of greater sensitivity in the etiolated coleoptiles, nor a retention of sensitivity until the curvature had become permanent, since those grown under red light were almost as sensitive to other filters as the ones grown in complete darkness. The effect was rendered more striking by setting the two series of coleoptiles alternately in the one tray within the apparatus to ensure similar conditions of the experiment, and finding them later alternately curved and vertical.

To ensure that difference in sensitivity was not the cause of the divergent result, the coleoptiles were exposed to blue light which had been weakened by neutral filters, until it had an efficiency value for etiolated coleoptiles equivalent to the green filter used. Both the coleoptiles grown in daylight and the etiolated ones remained permanently curved to this light. It was concluded that the green first leaf as it pierced the tip caused a diminution in response to stimulus when the coleoptile was exposed to green light. With the apparatus this could not be measured quantitatively by the method of opposing lights, since the time period was too short, nor was any effect shown on exposure to unilateral red light.

(vi) Though the coleoptile of *Avena* would not curve to red light whether grown in daylight or darkness, the coleoptile of *Zea mays* proved more suitable. Maize grown in sunlight curved readily to red light, and curved to a less extent to green. Etiolated maize coleoptiles curved readily to green light, and slightly to red light. But chlorophyll was always present, for if the temperature were kept so low that chlorophyll did not form, all growth ceased, and no phototropic curvature whatever took place. Maize requires a warm temperature in order to grow. As soon as the temperature was raised sufficiently for maize to grow, it also showed a tropic response to red light, though in some cases very little chlorophyll was present. It is probable, therefore, that maize, even if etiolated, would respond slightly to red light, but the response is definitely greater when chlorophyll is present.

(vii) The introduction of pigments to the *Avena* coleoptile artificially was next attempted, to see whether a curvature to red light could be induced. The coleoptiles were cut off above the seed, and stuck in a vertical position by using paraffin wax along the side of a square wooden rod (see Pl. III, Fig. 5). The wax gripped the coleoptiles sufficiently to prevent the first leaf from slipping out. They were then placed with the cut ends in a 5 per cent. cane sugar solution, and exposed to unilateral red light. No bending occurred in 40 hours, at the end of which time they were still sensitive to violet light of weak intensity.

Similar coleoptiles were now placed in a 5 per cent. cane sugar solution to which Azur-Eosin, a blue-green fluorescent stain, had been added in the proportion of 1 in 1000. They were exposed to red light for a period of 40 hours. By placing them with the narrower axis containing the vascular bundles of the coleoptile in the plane of the incident light, a slight curvature resulted in 33 out of 40 coleoptiles. The angle of curvature was up to seven degrees. (See Pl. III, Fig. 5.)

The curvature occurred in a manner directly opposite from the usual phototropic bending. It began low down in the coleoptile and spread upwards, though once curvature was effected in any region it remained permanent. The greatest curvature occurred in those coleoptiles in which the upward spread of stimulation was greatest. Once curvature had ceased, no amount of violet light had any phototropic effect, though prior to this, bending from the tip would occur in the normal manner.

Histological examination showed that in all cases where bending to violet light had ceased, the stain had penetrated the cells of the tip. The curvature to red light occurred in regions in which the stain had penetrated not far from the vascular bundle (which it did primarily by spreading out laterally), and yet was in sufficient concentration to impede light. The first leaf contained chlorophyll, and its bundles and surrounding lamina showed traces of the stain. It showed no signs of growth during the experiment, but since it was held at the base, moved downwards in the upper part of the coleoptile as the latter elongated.

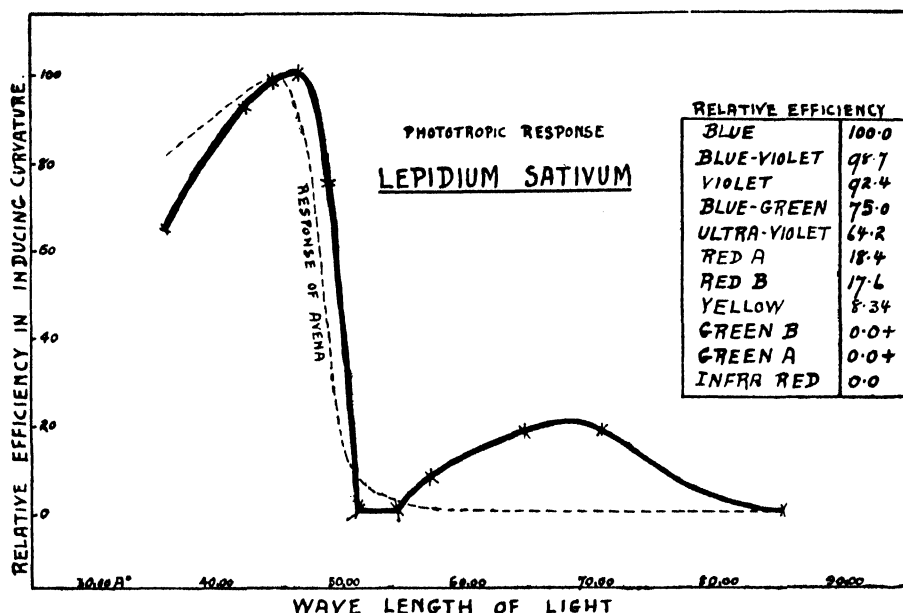
It seems possible that before the eosin stain prevented tropic response in the cells of the coleoptile (Boas (3)), a slight induced curvature took place in the lower regions. The power of curvature seemed to depend on a balance between the concentration of the stain, penetration of the stain outwards from the vascular bundle, and penetration of the tip.

#### *Results for Lepidium.*

The foregoing series of experiments seemed to show that the difference in response to the *Lepidium* hypocotyl grown in sunlight must be accepted, and the double series of experiments were conducted between opposing



lights in a similar manner to the *Avena* experiments previously described (see Pl. III, Fig. 6). The result for cress is expressed in the graph (see Text-fig. 5), and in comparison with oats, shows a marked efficiency in the red end of the spectrum, and a displacement of the maximum from blue-violet to blue. The response to green was so slight that it could not be quantitatively



TEXT-FIG. 5. Graph of phototropic response of *Lepidium*.

expressed. To determine the point of maximum efficiency in the red more precisely, two new filters (Red A and Red B) were used, showing that it lay between 6450 and 7060 Å. The maximum in the red region approximates closely to the chlorophyll absorption band in this part of the spectrum. A more refined method, using narrower spectral bands would probably give a higher value for efficiency in this region of the spectrum, as well as determining the position of the maxima more closely.

(c) *Celosia cristata*. In the work on *Lepidium*, the responses of *Helianthus annuus* had also been examined and found to be similar. A variety which produced large quantities of red anthocyanin pigment in the hypocotyl when exposed to strong sunlight was now investigated. Its responses were found to be much more sluggish than the green variety, and negative curvatures occurred very readily when the intensity of light was raised. This seemed to be a thermotropic reaction. Nevertheless the red *Helianthus* responded to red light in the same manner as cress, but had a lessened efficiency in blue, violet, and especially ultra-violet. No photo-

tropic response occurred in green light, but a negative thermotropic curvature was obtained very easily in this region of the spectrum.

Histological examination showed the red pigment present in the cells of the epidermis only, and it was apparent that this was acting as a filter to the incident light, the reduced quantities of light which penetrated behaving thereafter exactly as in a green plant. Red light entered easily, but blue and violet light were intercepted. Ultra-violet was absorbed especially, and it is probable in this lies the value of the pigment to the plant. The absorption of light by the pigment layer seemed to give rise to the thermotropic curvatures noticed.

Other plants containing red pigment were now examined, but the sluggish response and low threshold of thermotropic reaction made them unsuitable for any detailed investigation. Some hypocotyls of *Beta vulgaris*, in which histological examination showed red pigment throughout the cortex, were found to respond slightly to green light, but it was not until the late spring weather permitted the growth of *Celosia cristata* (Scarlet cockscomb) that any results of value were obtained.

*Celosia* produces much red pigment both in the epidermis and cortex, and the growing point is especially well provided. The hypocotyl is quickly grown, small in size, and gave a distinct angle of curvature. But like all red plants, its responses to light were extremely sluggish, since it took about 48 hours to react to the strongest blue-violet filter.

An alteration in the usual method of exposure to the light filters now led to greatly increased sensitivity and made detailed investigation possible. The *Celosia* seedlings were taken at a very early stage, when the radicle had just turned downwards into the soil and when the short hypocotyl still lay horizontal, and set so that they lay at right angles to the unilateral light. Instead of the hypocotyl becoming vertical, it now took up a position at an angle from the vertical in the direction of the incident light. This was much better than allowing the hypocotyl to straighten and then submitting it to unilateral light, as its greatest sensitivity was at the early period. When *Avena* and *Lepidium* were treated in the same way their sensitivity was increased considerably, but otherwise their reactions were the same. *Celosia* has the advantage of a very small seed, so that the seed-coat persisting over the cotyledons (as sometimes occurred) did not shield the hypocotyls from light.

Cockscomb hypocotyls were exposed to the series of filters and showed a strong response to green, blue, and violet lights, and a weak response to red and ultra-violet. No reaction to yellow occurred. The time of exposure gave no indication of the efficiency of the wave-length used, since the hypocotyls would remain for 30 hours in the apparatus without alteration in position, and would then orientate themselves to light within two or three hours. That the sluggishness of the vertical response to unilateral light

was due to the epidermal pigmentation was supported by the fact that geotropic stimulation was normal, and the efficiency of the various filters could be gauged quite accurately by the angle of curvature which resulted from the balance of phototropic and geotropic forces. Occasional seedlings also lacked epidermal pigmentation while still retaining pigment in the cortex, and these showed exaggerated responses to light. Such only appeared rarely among etiolated plants, and had of course to be discarded in the quantitative experiments.

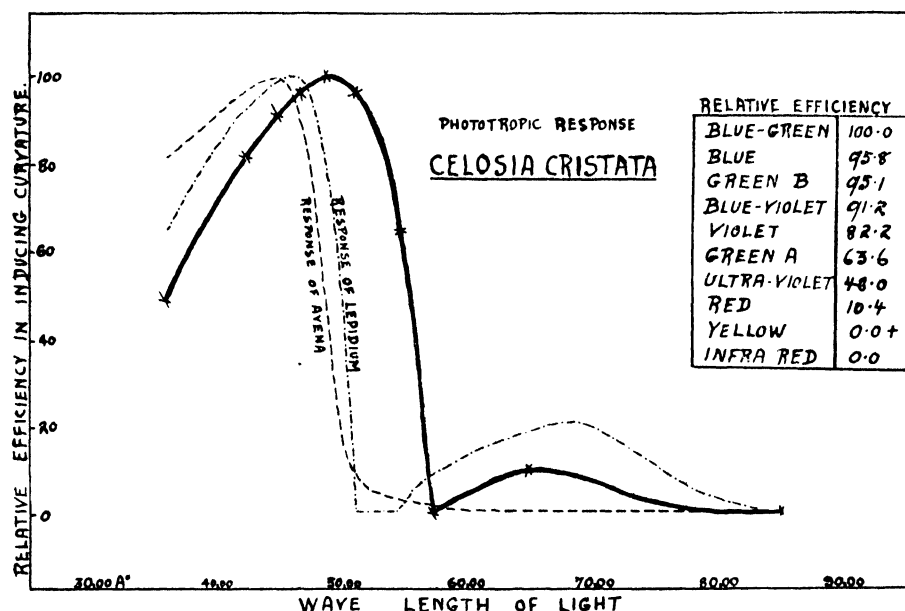
The hypocotyls which bent to red light contained chlorophyll, so further experiments were conducted in which the formation of chlorophyll was prevented by packing ice within the apparatus. This hindered but did not completely stop growth, and the hypocotyls became erect in 68 hours without any orientation to the red light. With violet light, weakened to the same efficiency for *Celosia* as the red, curvature at the low temperature took place in the normal manner.

An attempt was made to produce chlorophyll in the hypocotyls exposed to green light by illuminating them from above with red light while they were growing in the apparatus. The curvature to green light which ensued was much less than normal, but this may have resulted from the combination of red light plus gravity. The experiment was repeated and the red light removed as soon as the hypocotyls began to erect themselves, and again the response to green light was less than normal. But the effect of the red light may have persisted, or the production of chlorophyll may have hindered the production of red pigment, so that the value of the result was doubtful.

A careful series of experiments between opposing lights was now run to find the relative efficiency of the various filters (see Pl. III, Fig. 7), the results being graphed in the usual manner (see Text-fig. 6). The maximum response was now in the blue-green region, with a low response to ultra-violet and red. The shifting of the maximum towards the green region seems to be due to the red pigmentation, though it must be remembered that the pigmented epidermis acted as a preliminary filter and absorbed increasing amounts of green, blue, violet, and ultra-violet lights respectively without their being used in promoting curvature. Also the hypocotyls used were not entirely uniform, since those exposed to red and blue light contained chlorophyll, while those in green light did not. Why there was no response to yellow light can only be explained by assuming that the graph here tends to follow the *Avena* type of response, which is probably fundamental to plants unless modified by pigment content. The yellow filter had also the least energy value of the whole series, since it let through only a narrow band 200 Å wide.

To make quite certain that conditions were constant in both the cress and cockscomb experiments, a series of tests were conducted with *Lepidium*

and *Celosia* seedlings prior to the erect hypocotyl stage, in which they were planted alternately in the trays and exposed between green and red lights (see Pl. III, Fig. 8). The *Lepidium* hypocotyls grew towards the red, while the *Celosia* hypocotyls orientated themselves to the green light. Other similar experiments were conducted between blue-green and blue lights of



TEXT-FIG. 6. Graph of phototropic response of *Celosia*.

equal energy value, with a corresponding result. The responses were evidently of a different type.

## V. DISCUSSION.

In considering the lack of conformity in the results of previous investigators on the phototropic action of monochromatic light, it must be remembered that the conditions and methods of the various workers showed considerable divergencies. The aim of the present research was to overcome this as far as possible by standardizing the apparatus and testing a considerable range of plants. Though the results of only three plants, *Avena*, *Lepidium*, and *Celosia*, are given in detail, the number tested was considerably more, but no great difference was discovered which would warrant any detailed description of the others.

Considering, then, the responses of these three plants as typical of colourless, green, and red pigmented plants in general, we find various noteworthy features :

(a) The most striking differences occurred in the red and green parts

of the spectrum. This seemed directly due to the presence or absence of chlorophyll or anthocyanin pigment. However, as *Pilobolus* (Parr, 22) and *Zea mays*, though colourless, also respond slightly to red light, it seems necessary to postulate a more general hypothesis such as the light-gradient theory of Nuernbergk (21) as an explanation of phototropic reactions, and to accept the presence of pigment as causing an exaggeration or diminution of the light-gradient to certain wave-lengths of light. For full explanation of Nuernbergk's hypothesis, the reader is referred to his paper. The greater width of the coleoptile of *Zea*, the difference in response of the long and short transverse axes of the coleoptile of *Avena*, and the greater sensitivity of *Pilobolus* to all regions of the spectrum, together with the material here presented, seem adequate ground for the formulation of some such hypothesis.

(b) All the plants tested showed a diminution in response in the ultra-violet region. The light-gradient hypothesis would seem to demand that the response to ultra-violet should be greater than to blue-violet, since it is increasingly absorbed by the side of the plant nearest to the incident light. The present work on epidermal pigmentation and the consequent sluggish response of the plant to certain wave-lengths, may suggest that the actinic rays are increasingly absorbed by the epidermal layers, and consequently do not reach the light-sensitive area to the extent that their total absorption would indicate.

(c) If the results admit of some such interpretation as the above, it would be evident that phototropic curvature would be due to the initial stimulation of the light-sensitive region generally, rather than to a direct photochemical effect on some hormone or growth regulator. Castle's (4) suggestion that the graph of response is virtually an absorption spectrum of the substance responsible for phototropic curvatures must be discounted. Should the redistribution of auxin (Kogl, 14) be responsible for the curvatures, as the Went-Cholodny (25) hypothesis suggests, the process must be much more complicated than is generally suspected.

The writer desires to make further investigation into the relation of response in these two fields of research.

#### SUMMARY.

The need for a comparative study of a large range of plant responses to monochromatic light is stressed.

An apparatus is described which avoids the action of several variable factors and allows of a high degree of accuracy.

The response for *Avena* is in substantial agreement with the results of previous workers. The maximum response is about  $4480\text{ Å}$  with diminution in the ultra-violet and in the red. In red light only a slight effect is found.

With *Lepidium sativum* a different response is found, there being a second maximum in the red, and the initial maximum moves to about 4640 Å. in the blue.

In the absence of chlorophyll *Lepidium* gives the *Avena* type of response, and the nodal curvature of *Avena* at the second leaf stage, when chlorophyll is present, is similar to *Lepidium*.

Plants containing red anthocyanin pigment in the epidermis give a sluggish response, the pigment absorbing light without inducing curvature.

Presence of cortical pigmentation in *Celosia cristata* causes the initial maximum to move to 4860 Å. in blue-green.

A relationship is suggested between phototropic curvature and the light-gradient of the photosensitive area, rather than a direct photochemical effect on some growth-regulator.

The writer wishes to express appreciation and gratitude to Professor A. J. Ewart for criticism and encouragement during the development of this research, which was carried out in his department. He also wishes to thank Professor Hartung of the Chemistry Department and Mr. Rodgers of the Natural Philosophy Department for their interest and assistance in the composition of light filters and the control of the delicate instruments used in measurements of light.

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### EXPLANATION OF PLATE III

Illustrating Mr. Atkins's paper on 'The Effect of Pigment on Phototropic Response : A Comparative Study of Reactions to Monochromatic Light'.

- Fig. 1. *Avena* after exposure between two blue filters.
- Fig. 2. *Lepidium* bending to red light (4 hours' exposure).
- Fig. 3. *Avena* and *Lepidium* between red and green lights (red on right).
- Fig. 4. *Avena* bending at the node in red light (26 hours' exposure).
- Fig. 5. *Avena* containing azur-eosin in red light.
- Fig. 6. *Lepidium* between blue-violet and violet filters (blue-violet on the right).
- Fig. 7. *Celosia* between red and green A filters, after 80 hours (red on the left).
- Fig. 8. *Celosia* and *Lepidium* between red and green filters (red on the left).







## Studies in *Coprinus sphaerosporus*.

### III. The Inheritance of Factors affecting the Growth-rates at Different Temperatures of Certain Strains.

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With Plate IV and thirteen Figures in the Text.

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#### 1. INTRODUCTION.

IN the second paper of this series (5) an account was given of the inheritance of the morphological and physiological differences exhibited by certain haploid strains. In the present account these results have been extended by growing the haploids from a cross between two fast-growing strains, segregants from such a cross not having been previously examined.

Further information has also been obtained on the interactions of a haploid and a diploid colony when grown together on the same medium. The conclusions arrived at concerning the effect of the diploid on the haploid are in conformity with those expressed in the preceding paper of the series, though the avenue of approach is different. The converse effect, i.e. that of the haploid on the diploid, is not known to have been previously

observed. The present account, however, is chiefly concerned with the different growth-rates over a range of temperatures exhibited by certain strains and their progeny, and this has been applied not only to the haplo- but also to the diplo-phase. In the latter case it has been shown that a diploid having a growth-rate-temperature curve with a single peak at 30° C can give rise to diploids similar to itself, to others with a maximum growth-rate at 26° C., and to a third type which gives a bimodal curve with optima at 22° C. (major) and 26° C. (minor), respectively.

The inheritance of the 'sex' factors has also been determined in relation to those concerned with growth-rate.

## 2. EXPERIMENTAL PROCEDURE.

The technique employed is in all respects identical with that described in the preceding paper. Growth-rates were determined in dented test-tubes unless otherwise stated. The incubators used were adjusted so that the maximum range in temperature was never more than  $\pm 1^\circ$  C. and rarely greater than  $\pm 0.5^\circ$  C.

In the preceding paper (5) the strains which were crossed belonged to the 'C' generation; for the crosses recorded here, however, the following or 'D' generation has been used. The diploid (or mixture of diploids) from which this generation of haploids was derived arose in a mixed culture of the haploids C 30, C 21, C 10, and C 2. The haploids D 6 and D 14 were crossed in order to ascertain what  $F_1$  types were produced by two fast-growing strains. Strains D 1 and D 17 were found to react differently over a certain range of temperatures and were consequently crossed and their progeny examined in a similar manner. The  $F_1$  haploids of the cross D 1  $\times$  D 17 are referred to as the 'E' generation; certain of these were back-crossed with D 1 and others with strain D 20.

The method of referring to simple and compound diploids is similar to that previously employed. Thus  $D\ 17/E\ 17 \times D\ 1$  refers to the diploid arising from the diploidization of the haploid D 17 by the diploid  $E\ 17 \times D\ 1$ , and  $E\ 17 \times D\ 1/D\ 17$  refers to the 'rediploidization' (see below) of  $E\ 17 \times D\ 1$  by the haploid D 17. In Text-fig. 3 the figures underlined correspond to figures in italics in the text.

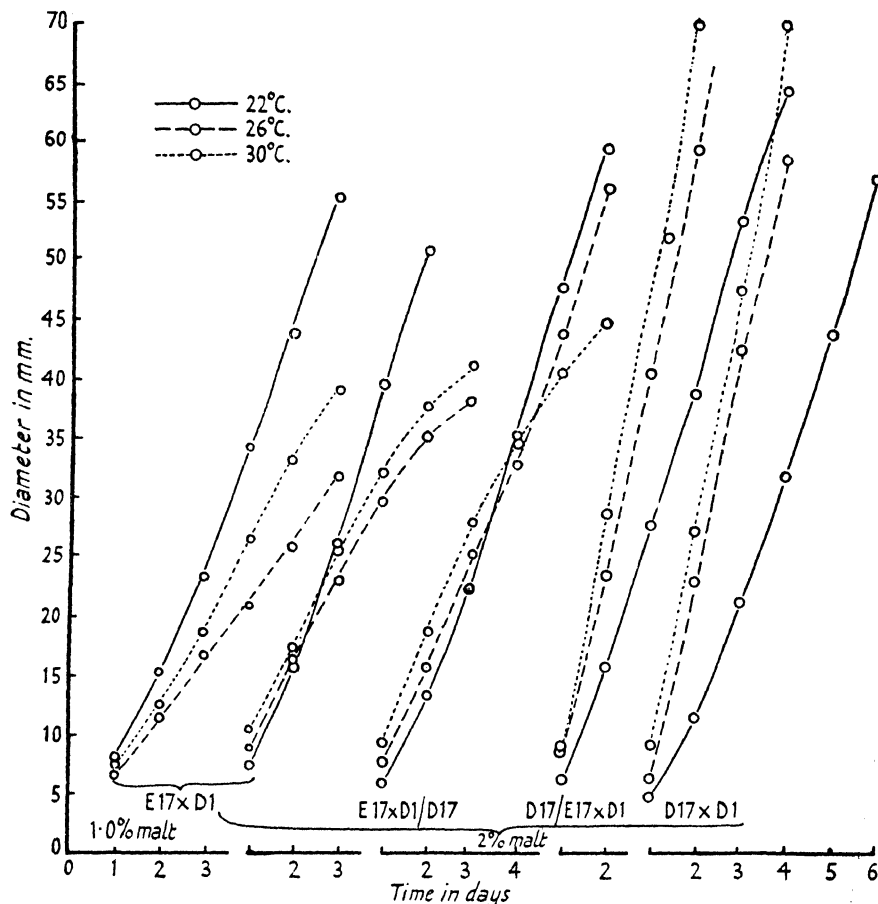
2% malt, 2% agar, was used throughout as the culture medium, and all measurements of growth-rate were made in duplicate unless otherwise stated.

## 3. THE EFFECT ON THE GROWTH-RATE OF A DIPLOID STRAIN OF PAIRING IT WITH A HAPLOID AND CONVERSELY.

In the first paper of this series (4) the growth-rates at 25° C. of four diploids were determined and it was found that they were all very similar. Subsequent work has shown that while probably the majority of a random

sample of diploids would have growth-rates similar to these, a certain number would show smaller growth-rates, the difference being most marked at 25–26° C.

Text-fig. 1 shows the rates of spread (increases in diameter) at 22, 26,

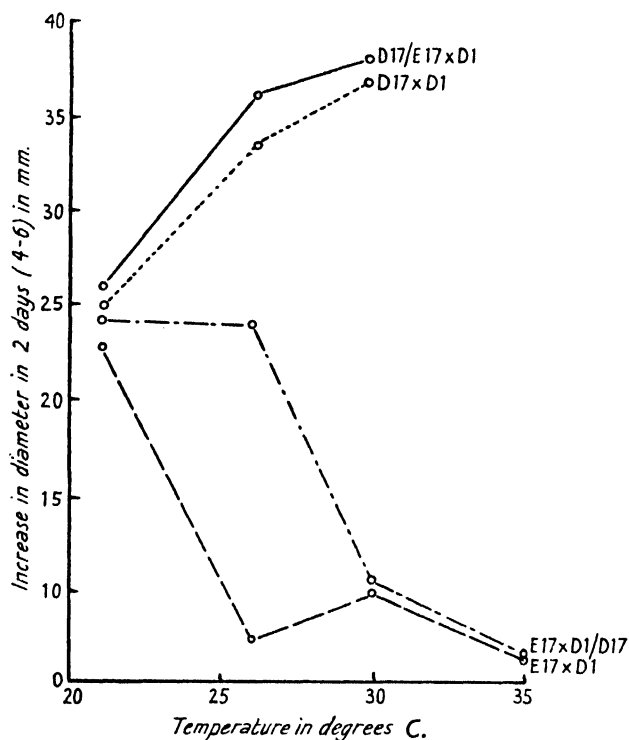


TEXT-FIG. 1. For explanation see text.

and 30° C. of strain D 17 × D 1 plotted against time. The rate at 26° C. is very similar to the rates at 25° C. of the four strains illustrated in Paper I. In the same figure the growth-rates of strain E 17 × D 1 (2% malt) are also given, and it can be seen that at 26 and 30° C. the rates of spread are much smaller than are those of strain D 17 × D 1. (It may here be pointed out that while strain D 1 is common to both diploids it is merely a coincidence that the number 17 should have occurred in each case as the two strains belong to successive generations.)

In Text-fig. 2 the rates of spread of D 17 × D 1 and E 17 × D 1 over a

period of two days (days 4-6) are plotted against temperature, which serves to accentuate the difference between them. In Part I reciprocal crosses of the fast diploids were shown to have very similar growth-rates and this has been subsequently verified in numerous cases. A similar result has been obtained for the slower diploids, and an example is given in Text-fig. 3

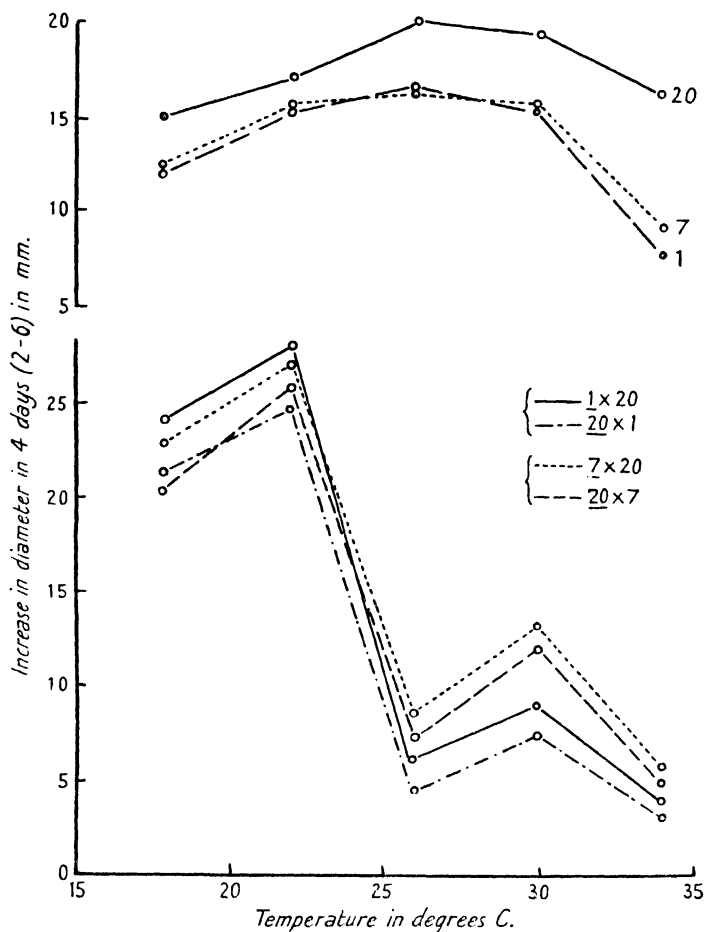


TEXT-FIG. 2. For explanation see text.

where the growth-rates of three haploids D 1, D 7, and D 20, and the reciprocal crosses resulting from their union in pairs, are plotted against temperature. It will be seen that the reciprocals are respectively very similar in their rates of spread at all temperatures. Since this was found to be the case it was not determined in the simple diploids D 17 x D 1 and E 17 x D 1, which reciprocal was being used.

s. It was thought possible that the differences in growth-rate at various temperatures of the diploids D 17 x D 1, and E 17 x D 1 might provide a new avenue of approach to the question of the interactions of diploids and haploids, and with this problem in mind the following procedure was adopted. The diploid E 17 x D 1 was paired in a Petri dish with the haploid D 17 (D 17 being of opposite sex to D 1), and after several days the latter strain was found to have been diploidized by the former. About a week after pairing

the two strains it was noticed that the periphery of  $E\ 17 \times D\ 1$  had produced a slightly more fluffy mycelium than the older part. Subcultures from the now diploidized  $D\ 17$ , i.e.  $D\ 17/E\ 17 \times D\ 1$ , and from the periphery of  $E\ 17 \times D\ 1$  at the point farthest removed from  $D\ 17$  were made. The latter



TEXT-FIG. 3. For explanation see text.

strain will be referred to as  $E\ 17 \times D\ 1/D\ 17$  to indicate that it has been in organic connexion with  $D\ 17$ . Strain  $E\ 17 \times D\ 1/D\ 17$  was then subcultured from a single large branch of mycelium. Strains  $E\ 17 \times D\ 1$ ,  $D\ 17 \times D\ 1$ ,  $D\ 17/E\ 17 \times D\ 1$ , and  $E\ 17 \times D\ 1/D\ 17$  were incubated respectively at both 22° and 26° C., and were allowed to grow for eight days, when they were recultured from the peripheries of the colonies. The cultures from the colonies grown at 22° C. were measured (in triplicate) for growth-rate at the temperatures 22, 26, and 30° C., those at 26° C. were re-incubated at this

temperature. Text-fig. 1 shows the growth-rates of all four strains plotted against time, and Text-fig. 2 their respective increases in diameter in two days (days 4-6 from the time of subculturing) plotted against temperature. In each figure it can be seen that strains  $D 17 \times D 1$  and  $D 17/E 17 \times D 1$  are very similar in growth-rate at each of the three temperatures respectively, while strains  $E 17 \times D 1$  and  $E 17 \times D 1/D 17$  differ considerably from them and from one another. All four strains have very similar growth-rates at  $22^{\circ}C.$ , and their rates of spread are practically constant for the first five days. Strains  $D 17/E 17 \times D 1$  and  $D 17 \times D 1$  show increasing growth-rate with rising temperatures, the growth-rate at any one temperature being practically constant over a period of 4 or 5 days. Strain  $E 17 \times D 1/D 17$  has almost identical and constant growth-rates at  $22$  and  $26^{\circ}C.$ , but at  $30^{\circ}C.$  the rate of spread falls off with time. The growth-rate of strain  $E 17 \times D 1$  is greatest at  $22^{\circ}C.$ , least at  $26^{\circ}C.$ , and intermediate between these two (but nearer that at  $26^{\circ}C.$ ) at  $30^{\circ}C.$

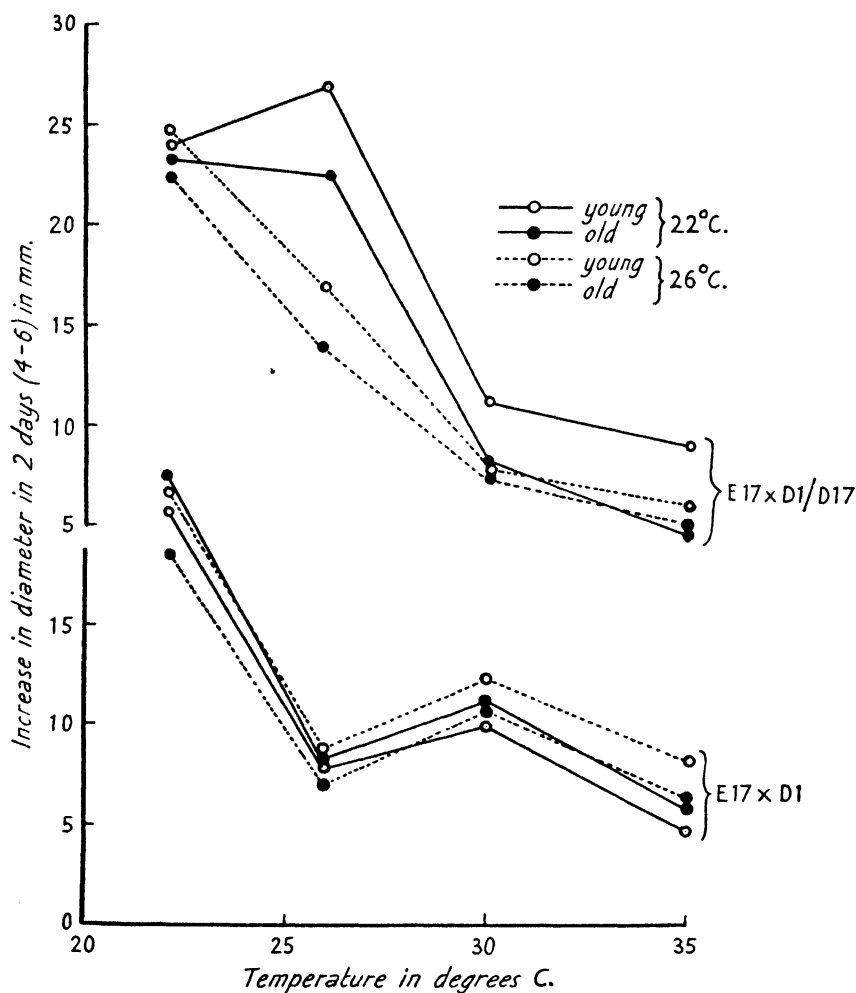
From these figures it will be seen that  $E 17 \times D 1/D 17$  is in certain respects intermediate between  $E 17 \times D 1$  and  $D 17 \times D 1$ , and this is supported by the morphology, as at each temperature  $D 17 \times D 1$  produces a more plentiful aerial mycelium than  $E 17 \times D 1$ , and  $E 17 \times D 1/D 17$  at the same temperature is approximately intermediate between them.

The cultures of strains  $E 17 \times D 1$  and  $E 17 \times D 1/D 17$  used for measurement and which had been grown at  $22^{\circ}C.$  and those which were originally and continuously incubated at  $26^{\circ}C.$  were allowed to grow at these temperatures until they were ten days old, when subcultures were taken from the young and old parts of each colony and used to determine growth-rates, the object being to ascertain the effects of age and temperature on growth-rate. Text-fig. 4 shows the results of the measurements. The rate of spread in two days (days 4-6) is in each case plotted against temperature, the curves of the two strains being separated for convenience.

It will be seen that in the diploid  $E 17 \times D 1$  all the curves have the same form and the growth-rates at any one temperature are very similar in magnitude. It is concluded from this that neither the temperature at which the strain was previously grown nor the age of the mycelium used for the subcultures has any appreciable effect on the rate of spread of this strain at the different temperatures respectively. The mycelial characters at each temperature were similar irrespective of previous treatment of the inoculum. The four different treatments of the inocula of strain  $E 17 \times D 1/D 17$  have produced in contrast to the results with strain  $E 17 \times D 1$  different curves in each case, and in no instance is there a curve similar to those of the latter strain.

The chief difference between the curves is found in the growth-rates at  $26^{\circ}C.$  At this temperature all growth-rates are higher than in strain  $E 17 \times D 1$ ; the smallest rate of spread is found in mycelia grown from old

inocula incubated at 26° C., and the fastest from young inocula at 22° C. It therefore appears that previous incubation at 22° C. causes a greater difference between the two diploids ( $E 17 \times D 1$  and  $E 17 \times D 1/D 17$ ) than incubation at 26° C., and that at either temperature the difference is more



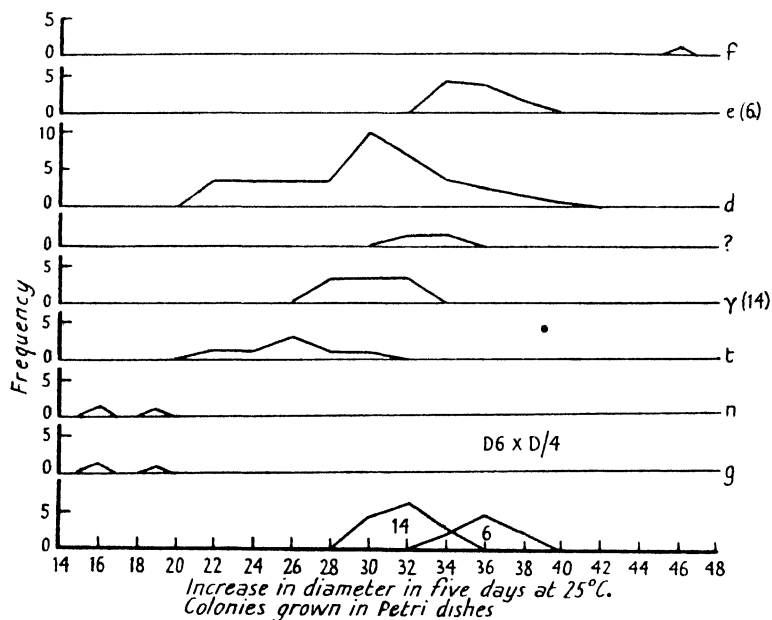
TEXT-FIG. 4. For explanation see text.

pronounced in young than in old subcultures. The difference between the two diploids (at 22° C. and with young inocula) increases also with time. This can be seen on comparing the growth-rates of the mycelium of  $E 17 \times D 1/D 17$  derived from young inocula incubated at 22° C. (Text-fig. 4) with that of the same diploid (Text-fig. 2) which was derived from young inocula grown at the same temperature, but which was measured ten days previously.



4. SEGREGATION IN THE CROSS  $D6 \times D14$ .

The growth-rates of the haploid segregants from the cross  $D6 \times D14$  were measured. The cultures were then arranged in groups, the members of each group having similar morphological characters, and frequency distributions of each group were prepared. These are shown in Text-fig. 5.



TEXT-FIG. 5. For explanation see text.

Altogether there were eight different types, of which six were established as being similar to types found among the segregants described in Part II of this series. One of the remaining two types could not be placed with certainty as the original types were no longer available for comparison (it is indicated by a question-mark in the figure), the other was very similar to strain number 5 in Part I, and will be referred to as  $\gamma$ . Strain 5 gave rise to diploid colonies on being X-rayed, but though three different colonies of type  $\gamma$  were irradiated no such effect was obtained. The relative frequencies of the eight types were as follows: type  $d$  56,  $e$  14,  $f$  1,  $g$  2,  $n$  3,  $t$  9,  $?$  3, and  $\gamma$  12. The parent  $D6$  was identical with type  $e$  and  $D14$  with type  $\gamma$ , so that type  $d$ , which is not a parental type, occurs in the  $F_1$  with a much greater frequency than that of either of the parental types.

The frequency distributions of all the segregants taken together from the above cross, from a cross  $D1 \times D17$  to be referred to in the following section, and from the six simple crosses described in the preceding paper of the series are shown in Text-fig. 6. Growth-rate determinations in the

cross D 6 × D 14 were made on Petri dishes, whereas in all the other crosses dented test-tubes were used. This difference in technique may cause a small alteration in the absolute growth-rates of the strains when compared with their rates of spread in test-tubes, but the relative positions of the different strains should be approximately the same (*vide* Part II, p. 183). The growth-rate at the mode of each parental strain is indicated by a vertical dotted line, to the right of which is the number of the strain. Still farther to the right is a letter in brackets, indicating to which type the parent belongs. The types to which strains 2, 15, and 17 belong were not found in any of the  $F_1$  segregants, and no type-letter has therefore been assigned to them. The growth-rate at the mode of each segregating type in each cross is indicated by the position of the reference letter of the type. Thus in the cross C 21 × C 30 the letter *d* indicates that the modal growth-rate of type *d* segregants in this cross was approximately 26 mm. A comparison of the growth-rates of any one type segregating from the different crosses shows them to be very similar, the greatest discrepancy being in type *t* segregating from the crosses C 10 × C 21 and D 6 × D 14. This discrepancy may be due to a differential effect on the growth-rate of type *t* arising from its culture in one instance in Petri dishes, and in the other in test-tubes, and its irregular manner of growth involving difficulty in measurement may also be to some extent responsible. The latter hypothesis is supported by the wide deviation from the mean to be seen in Text-fig. 5, and more markedly in Text-figs. 10 and 11 of Part II.

It will also be seen that irrespective of the parental growth-rates the range of growth-rates of the segregants from any cross is very similar to the range in any other cross.

The percentages of 'dwarf' colonies, i.e. those with limited growth, in the segregants from each cross are also shown on the figure.

Table I is a modified form of Table I in Part II of the series. The compound diploids have been removed for convenience, and the results of the cross D 6 × D 14 added. The 'group' letters shown in the table will be referred to in the following section of this paper. The 'type' letters of the parental strains are also given—strains 2, 15, and 17 do not occur among the segregants, and consequently have no type-letter, the spaces are therefore filled with question-marks. Of the segregants, those in italic type are similar to one or other parent. The relative frequencies of types *d* and *e* have been slightly altered from those presented in the original table. As explained in the preceding paper, types *d* and *e* are difficult to separate morphologically as there are numerous intergrading types, and for that reason their frequencies were in some cases grouped together. In the table presented here they have been separated arbitrarily into two groups, those having a growth-rate of over 30 mm. being grouped as type *e* and those under 30 mm. as type *d*. From the frequency distributions of the two

types taken together this has proved satisfactory in each cross, and a comparison of the figures with those in the original table (where the separation was based on morphological grounds only) shows that only in the segregants of the cross  $21 \times 30$  is the difference at all important.

TABLE I.

Group	A B	B B	B C	A B	A C	C C	A A
Type	e ?	d ?	h d	e d	e ?	h ?	$\gamma$ e
Cross	$30 \times 17$	$16 \times 15$	$10 \times 21$	$30 \times 21$	$30 \times 2$	$10 \times 2$	$14 \times 6$
	a 28	a 23					
	b 5						
	c 12						
	d 16	d 11	d 21	d 27	d 13	d 22	d 56
	e 39	e 22	e 22	e 63	e 46	e 18	e 14
	f 1					f 5	f 1
Segregants			g 8			h 6	g 2
		i 25	h 22				
				j 2			
				k 2			
				l 2			
				m 1			
			n 19				n 3
					o 8		
					p 31		
	q 15						
				r 3			
	s 3		t 7				
						u 4	t 9
						v 6	
						w 3	
						x 23	
						y 5	
						$\beta$ 8	
							$\gamma$ 12

From the table it will be seen that in the following instances a segregant type different from either parental type occurs with a greater frequency than that of either parent.

$30 \times 17$ . The parental type 17 does not appear in the segregants.

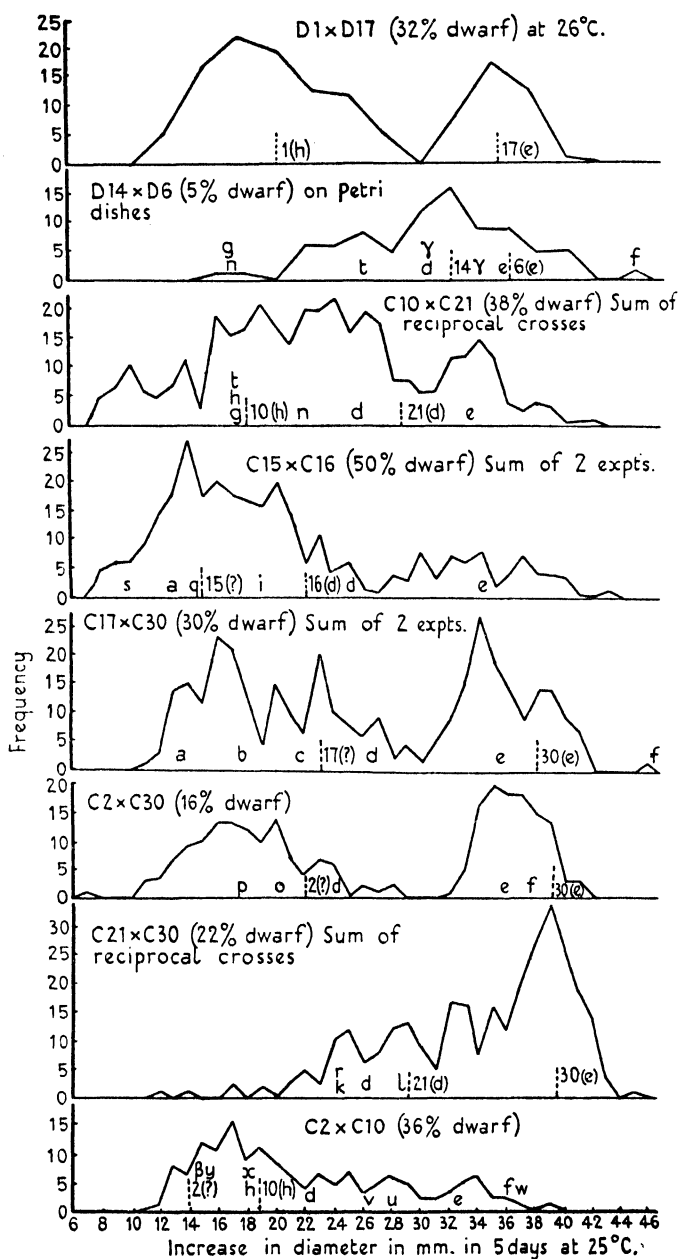
$16 \times 15$ . Types *a*, *e*, *i*, and *q* have frequencies of 23, 22, 25, and 15, respectively, compared with that of 12 for the parental type *d*. The other parental type appears not at all among the segregants.

$10 \times 21$ . Type *e* has a frequency of 22 in comparison with 21 for the parental type *d*.

$30 \times 2$ . The parental type 2 does not occur among the segregants.

$10 \times 2$ . Here, again, type 2 is absent in the  $F_1$  haploids, and types *d*, *e*, *x*, and  $\beta$  occur with greater frequencies than the other parent, type *h*.

$14 \times 6$ . Type *d* occurs with a greater frequency (56) than either of the parental types *e* (14) and  $\gamma$  (12).



TEXT-FIG. 6. For explanation see text.

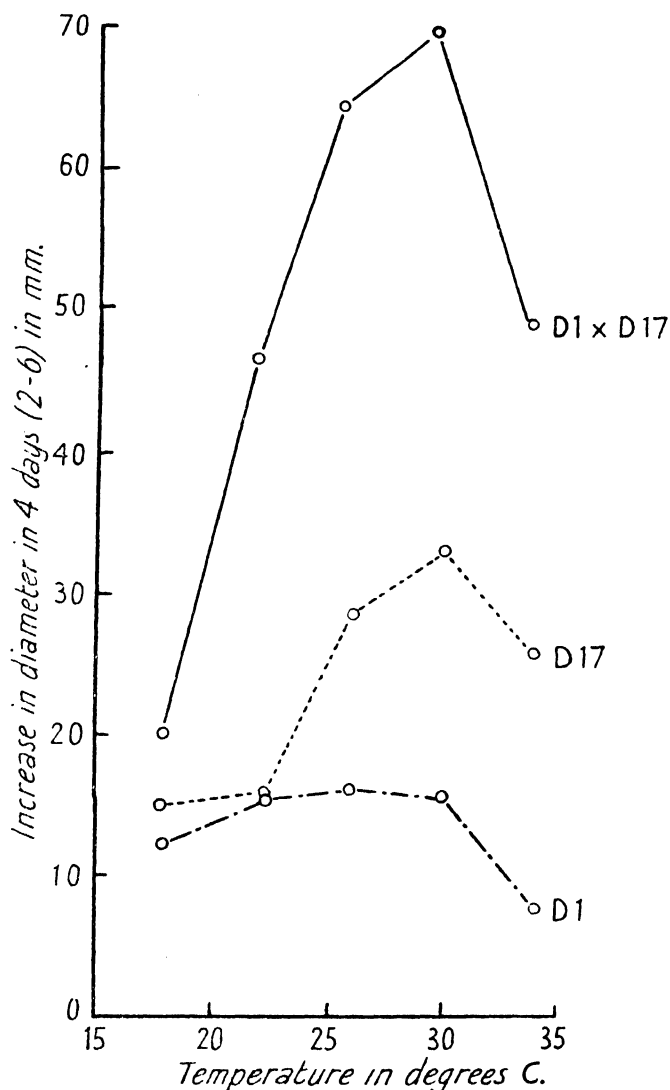
5. SEGREGATION OF 'SEX' AND GROWTH-RATE IN THE HAPLOID  $F_1$  OF A CROSS BETWEEN STRAINS D 1 AND D 17 WHICH RESPONDED DIFFERENTLY TO CERTAIN TEMPERATURE CHANGES.

The two strains D 1 and D 17 were selected owing to their marked differences in growth-rate at certain temperatures. Text-fig. 7 shows the growth-rates of the two strains at five different temperatures, namely, 18, 22, 26, 30, and 34° C. It will be seen that while the growth-rates of the two strains are very similar at 18 and 22° C., they differ markedly at the three higher temperatures. Strain D 1 shows a slight increase in growth-rate from 18 to 22°, then it remains practically constant from 22 to 30°, when it falls off fairly rapidly. In strain D 17, on the other hand, the growth-rate rises slowly from 18 to 22° C., and then rapidly from 22 to 30° C., finally decreasing to 34° C. The growth-rate of D 17 is, then, much greater between 26 and 30° C. (about twice as rapid) than is that of D 1, and at the same time the curve has a well-defined peak at 30° C., whereas in D 1 there is no sharp optimum temperature. Strain D 17 at 26° C. produces a plentiful fluffy aerial mycelium, and in both its growth-rate and appearance is similar to type  $\epsilon$ . D 1, on the other hand, gives a very thin aerial mycelium, evenly distributed, and a colony with a very even edge. On crossing these strains the diploid D 1  $\times$  D 17 was produced. Its growth-rate is shown in Text-fig. 7, and it will be seen that it produces a curve with a sharp maximum point at 30° C. above, and below which temperature the growth-rate rapidly decreases.

Of 267 single-spore colonies derived from a fruit-body of the diploid D 1  $\times$  D 17, 82, or 31 per cent., were dwarf. The growth-rates of the remaining 185 were determined at each of the temperatures 18, 22, 26, 30, and 34° C. Of the 185 colonies, 128 were measured in duplicate and 57 with one measurement for each temperature. While single cultures were adequate for determining the morphology of the colonies and for establishing a rough idea of their growth-rates at the different temperatures, the measurements were obviously not so reliable as those obtained when the colonies were grown in duplicate. In preparing the frequency distributions shown in Text-fig. 8 only those colonies measured in duplicate have therefore been included. The frequency curve at the top of Text-fig. 8 shows the growth-rates of 128 haploids from the cross D 1  $\times$  D 17. The dotted vertical lines represent the growth-rates at the modes of the frequency curves of D 1 and D 17. The growth-rates were taken as the increases in diameter of the respective colonies in four days (days 2–6) at 30° C. A comparison of this curve with that for the same strains grown at 26° C., and calculated for five days' growth (Text-fig. 6), shows apart from certain minor differences a fairly close similarity between them.

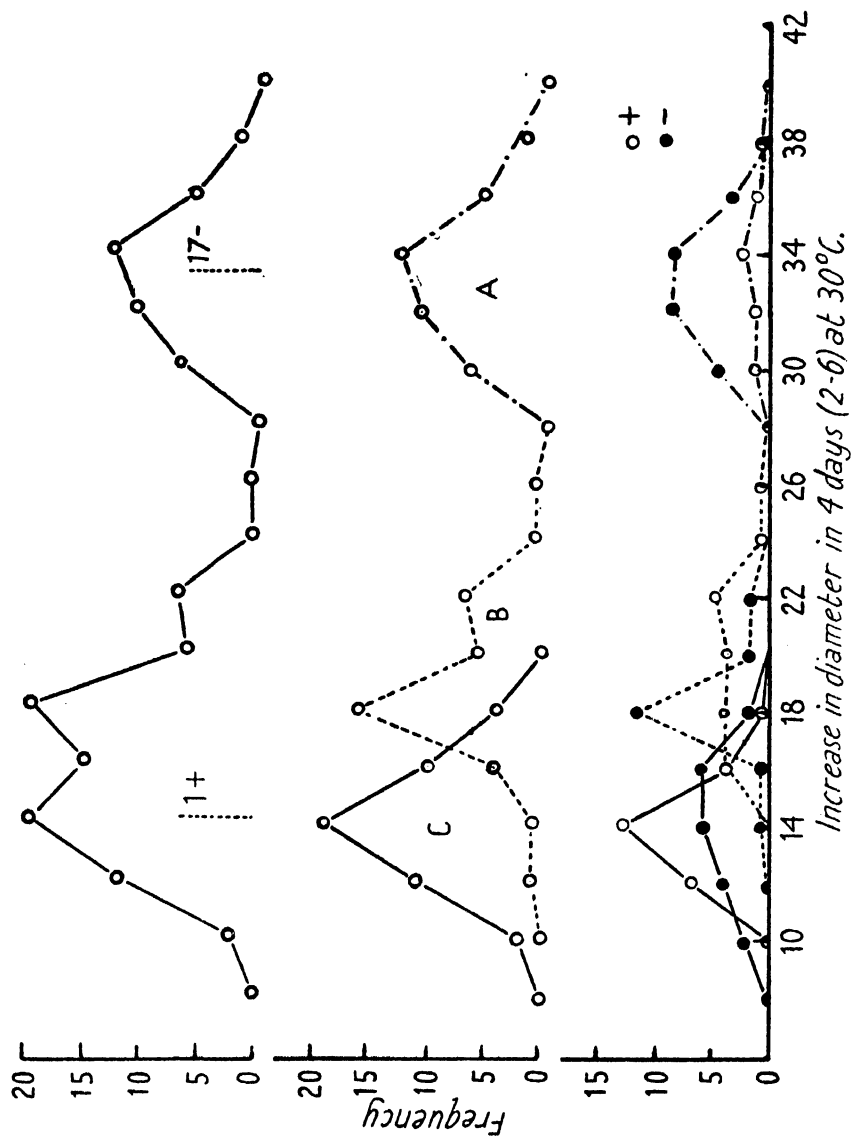
The strains were then separated into the 'groups' on morphological

grounds. These are the groups A, B, and C, to which reference has been made in regard to Table I. Group A consisted of type *e* colonies only. Colonies of type *d* were few in number, and in each case readily separable



TEXT-FIG. 7. For explanation see text.

in both appearance and growth-rate from those of type *e*. Group B contained all colonies whose mycelia were at all irregularly distributed over the surfaces of the media and all those in which the aerial mycelium could be described as plentiful. In this group were included the few type *d*



TEXT-FIG. 8. For explanation see text.

colonies which were produced among the segregants. Group C contained all the remaining strains, i.e. those whose aerial mycelia were thin and evenly disposed over the surface of the colony. The separation was based on the morphology exhibited by the colonies at 26° C. Frequency distributions of the three groups are shown in the middle graph of Text-fig. 8, and it can be seen that each group forms a fairly normal frequency curve. At the bottom of the same figure frequency distributions of the two sexes in each group are given, the distribution of each sex agreeing fairly closely with that of the other sex in the same group. The chief difference here is found in group B where the + strains have on the whole a wider deviation and a higher mean growth-rate than the – strains.

The numbers of segregants found in each group were as follows:—Group A 51, group B 54, group C 80, and dwarfs 82. Of these strains, 38 in group A, 39 in group B, and 72 in group C were selected at random, and tested for sex by back-crossing with the two parents D 1 and D 17. The results are given in Table II. The ratios obtained in each group and the values of P obtained from Fisher's Table of  $\chi^2$  (7) are as follows:—Group A 12+26–, P 0.2–0.5, group B, 24+15–, P 0.1–0.2, group C 38+34–, P 0.5–0.7. It will thus be seen that while the numbers in groups B and C are not significantly different from a 1:1 ratio the difference in group A is significant.

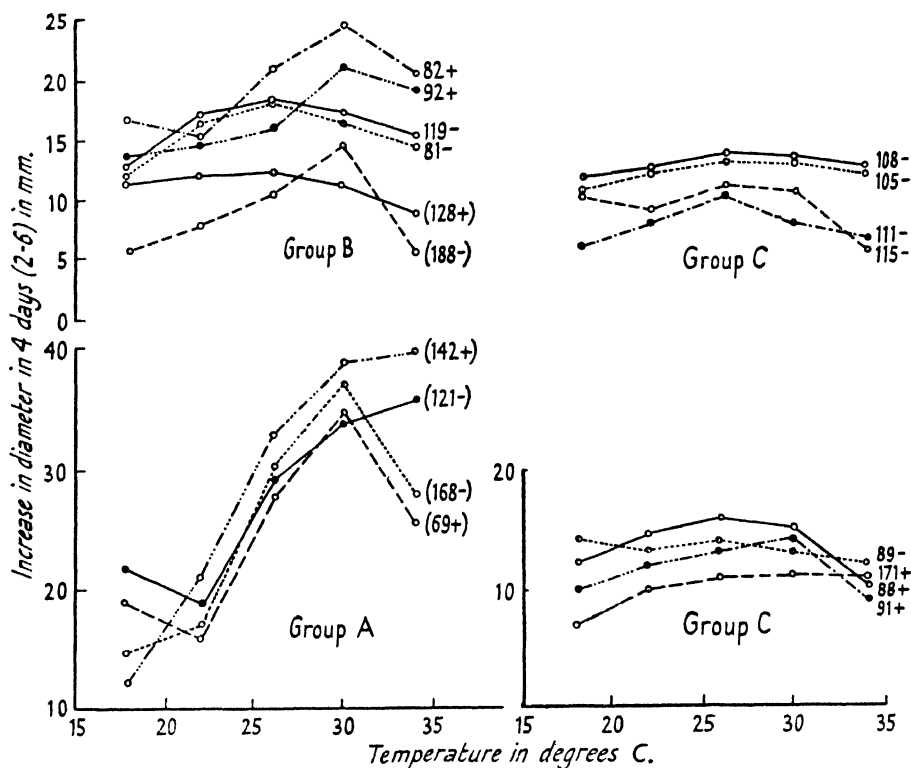
Text-fig. 9 shows the growth-rates of selected strains of groups A, B, and C in 4 days at 5 temperatures. Of the 185 strains whose growth-rates were determined only 18 are shown. These do not represent a random sample from each group, but have been selected to show all types, including extreme forms.

Group A are all of the same type as D 17 (Text-fig. 7), though certain strains (9 out of 51 measured), e.g. 121 and 142, show a small increase in growth-rate with a rise in temperature from 30 to 34° C., whereas in strain D 17 the rate falls off at temperatures above 30° C. Other examples showed a smaller decrease in growth-rate above 30° C. than that found in D 17. In all cases, however, there is a sharp increase in growth-rate between 22 and 30° C., and above that temperature in the majority of strains it falls off equally rapidly. A type *f* colony did not appear among the segregants, but as its growth-rate is greater than that of type *e* it was thought of interest to measure it at different temperatures. This was done and it was found to respond similarly to colonies of the B group.

Group B show a wide range of types, some having maximum growth-rates at 26°, and others at 30° C. In a few the peak is well marked, e.g. strains 82, 92, and 188, while others show only a small variation in growth-rate with changes in temperature, e.g. strain 128. It will be seen that the former type has 30° C. as the peak temperature and the latter 26° C., and this applies to all the strains of group B.



*Group C.* The two groups of 4 strains each have been separated in the Text-figure for convenience. These strains differ from those of group B firstly, in being very similar to one another, and secondly, in having a smaller average growth-rate. The response to temperature changes is in



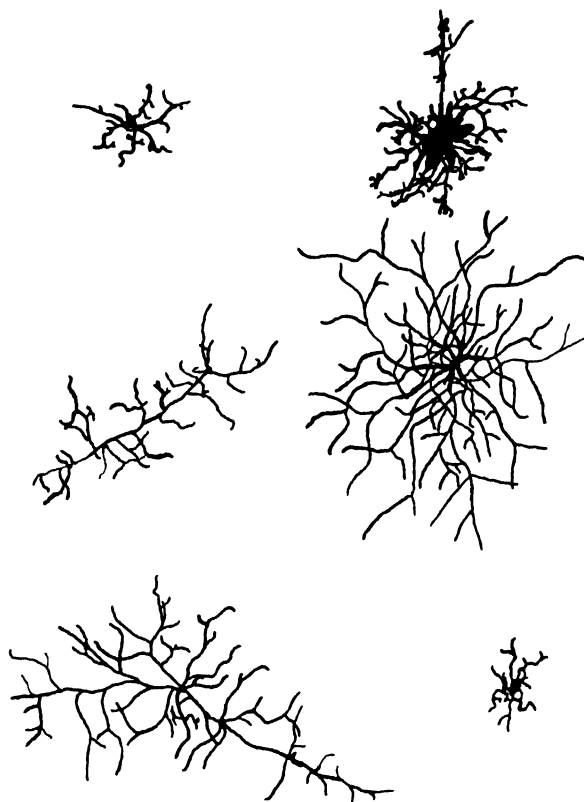
TEXT-FIG. 9. For explanation see text.

all cases small, and in most strains the temperature of maximum growth is 26° C.—strain 91 + is a rare exception. From the foregoing it would appear that groups A and C are fairly homogeneous, whereas group B shows considerable variability.

It will be seen from these data that group B is in some respects intermediate between groups A and C. Apart, however, from the few exceptional cases, to which reference has already been made, the similarity between B and C is much greater than that between groups B and A.

Morphologically the strains varied considerably in their response to increasing temperatures. Generally, however, the maximum amount of mycelium was formed at 26 and 30° C. Staling, which occurred in a mild form, and only in some members of group B, took place at 26, 30, and 34° C., and never at 18 and 22° C. A frequency distribution of the staling strains

of type B (at 30° C.) was very similar in mean value and deviation to one formed from the non-staling members. It will be seen in all three groups, and especially in group A, that some strains have weakly defined minimal growth-rates at 22° C., e.g. strains 69 and 121 of group A, 82 in group B, and 89 and 115 in group C. While this is only so in some strains, it was



TEXT-FIG. 10. For explanation see text.

found that in every case at a temperature of 22° C. the amount of aerial mycelium was less than that at 18 and 26° C. It is probable that these results are related despite the fact that the physiological effect is not always apparent.

Pl. IV, Figs. 1–7 show seven haploids grown for four days at the 5 temperatures (from left to right) of 18, 22, 26, 30, and 34° C. Figs. 1 and 2 show type *e* colonies of group A, Figs. 3–5 various group B colonies (Fig. 3 is of type *d*), and Figs. 6 and 7 of group C. It can be seen by a comparison of Fig. 3 with Figs. 1 and 2 that while type *d* is fairly similar in appearance to type *e* (except at 34° C.), it is quite different from the latter in its rate of spread at the different temperatures. In every case (though

the effect is not well marked in group C, Figs. 6 and 7) the amount of aerial mycelium is less at 22° than at 18 or 26° C.

Text-fig. 10 shows free-hand drawings (magnification approximately  $\times 70$ ) of six dwarf colonies, four days from the time of sowing the spores. They have reached about their maximum size. It will be seen that whereas the three larger colonies are fairly normal in appearance, the smaller ones are of definitely aberrant types. The smallest non-dwarf colony found had about three times the diameter of the largest dwarf shown.

TABLE II.

Haploid Parents		D 1 + (Group C)	×	D 17 – (Group A)	
Diploid		D 1	×	D 17	
Haploid F 1	Dwarf	Group C		Group B	Group A
	82	80		54	51
No. tested for sex		72		39	38
Sex ratios found (total 74+ : 75–)		38+	34–	24+	15–
		×		×	×
		D 20 – (Group B)	D 1 + (Group C)	D 20 – (Group B)	D 1 + (Group C)
Diploids					×
					Colonies of Groups A, B, and C
Series $\alpha$		31	8	22	7
„ $\beta$		3	26	2	6
„ $\gamma$		4	0	0	2
					0
					0
					25

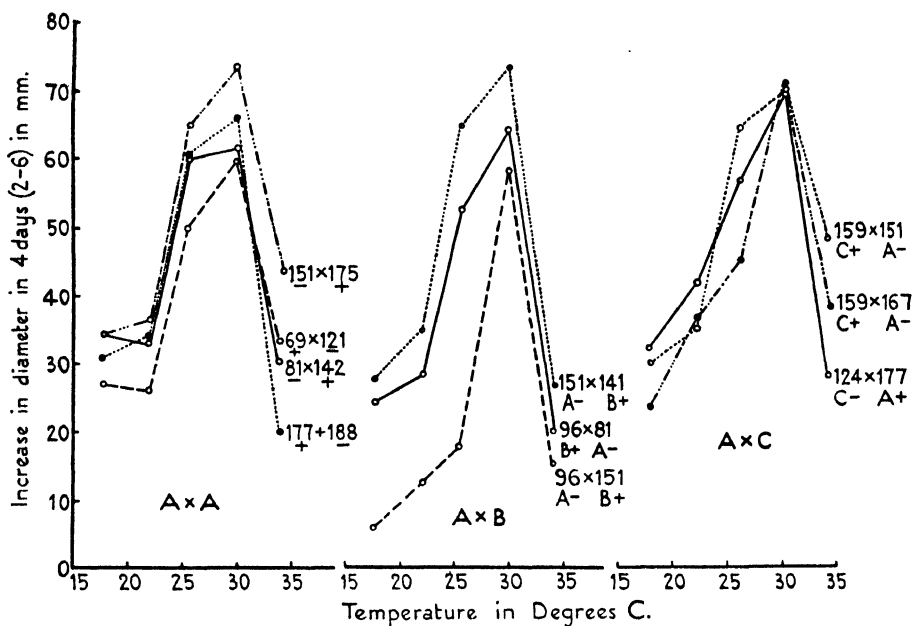
## 6. ABSOLUTE GROWTH-RATES AND THE TEMPERATURES OF MAXIMUM GROWTH-RATE OF CERTAIN DIPLOID STRAINS.

Morphologically the diploids show less variation with temperature changes than do the haploids. Many exhibit a decrease in the amount of aerial mycelium at 22° C. (as is the case with the haploids), but this is not always so. In most strains the mycelium is at a maximum at 26 to 30° C., and while generally it is evenly dispersed, in certain of the diploids (not produced by the use of a group A haploid) a certain amount of irregularity is found at 26° C. and upwards. Pl. IV, Fig. 8 shows a C  $\times$  C diploid in which this is exceptionally clear. The reduced aerial mycelium at 22° C. can also be seen in this figure.

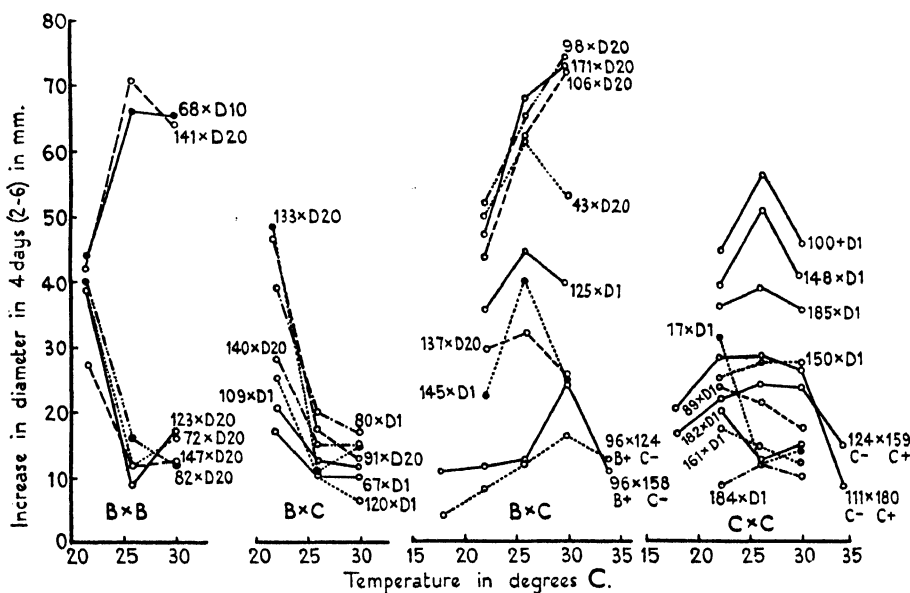
Text-figs. 11 and 12 show the growth-rates at different temperatures of certain diploid strains derived from the union of members of the following groups:—A  $\times$  A, A  $\times$  B, A  $\times$  C, B  $\times$  B, B  $\times$  C, and C  $\times$  C. As in the case of the haploids, the examples shown constitute a representative rather than a random sample. The diploids A  $\times$  A, A  $\times$  B, and A  $\times$  C, while showing individual differences, are all of a common type. In all cases the temperature of maximum growth-rate is 30° C., above and below which

point the rate falls rapidly; the rate of spread at this temperature lies between 60 and 75 mm. The diploid  $96 \times 151$  in the  $A \times B$  class differs most from the average in that its growth-rates at 18, 22, and  $26^{\circ}\text{C}$ . are abnormally small. Altogether thirteen  $A \times B$  diploids have been measured, and as this is the only example of the type it is probable that it is of rare occurrence. As these three classes of diploids have all a parent of group A (type  $e$ ) in common, while the other parent differs greatly from class to class (and intra class in classes  $A \times B$  and  $A \times C$ ) and are all of a common type (series  $\gamma$ ), it must be concluded that haploids of group A have a dominating influence on the growth-rate of any diploid to which they give rise.

Compared with the three classes of Text-fig. 11, those in Text-fig. 12, namely,  $B \times B$ ,  $B \times C$ , and  $C \times C$ , reveal wide variations, not only inter, but intra class. Class  $B \times B$  consists of two types, those in which the growth-rate rises to a maximum at  $26^{\circ}\text{C}$ . (series  $\beta$ ), and those in which it falls rapidly at temperatures immediately above  $22^{\circ}\text{C}$ . (series  $\alpha$ ). There are numerous minor variations of the latter type. In some strains, e.g.  $123 \times D 20$ , the growth-rate is a minimum at  $26^{\circ}\text{C}$ ., after which it rises again; in others, e.g.  $82 \times D 20$ , it continues to fall to  $30^{\circ}\text{C}$ . In only four diploids of series  $\alpha$  have the measurements been repeated, and in each case the results were in agreement with those first obtained, namely, a small increase in rate between 26 and  $30^{\circ}\text{C}$ . That the slow rate of spread at  $26^{\circ}$  is not due to staling is seen in Text-fig. 1 for the strain  $E 17 \times D 1$  grown on 1% malt. In this case the growth-rates at each of the temperatures 22, 26, and  $30^{\circ}\text{C}$ . are approximately constant from the first to the sixth day, the maximum rate being at  $22^{\circ}\text{C}$ . and the minimum at  $26^{\circ}\text{C}$ . It should be noted that  $\beta$  series types have a growth-rate at  $26^{\circ}\text{C}$ . comparable with that at  $30^{\circ}\text{C}$ . for the  $\gamma$  series. In the  $B \times C$  class the series  $\alpha$ ,  $\beta$ , and  $\gamma$  are all to be found. The  $\gamma$  types have growth-rates at  $30^{\circ}\text{C}$ . comparable with those in classes  $A \times A$ ,  $A \times B$ , and  $A \times C$ , and also a few examples, e.g.  $96 \times 158$  and  $96 \times 124$ , in which the rates of spread are much smaller. The  $\beta$  series also shows a wider range of growth-rates in types  $43 \times D 20$ ,  $125 \times D 1$ , and  $137 \times D 20$  than has been found in the preceding classes. The class  $C \times C$  has no very high growth-rates. Some of the  $\alpha$  series in this class are notable in having relatively slow growth-rates at  $22^{\circ}\text{C}$ . when compared with those of classes  $B \times B$  and  $B \times C$ . The  $\beta$  series has a wider range of growth-rates at  $26^{\circ}\text{C}$ . than it has in class  $B \times C$  as it varies from 60 mm. in type  $100 \times D 1$  to 10 mm., approximately, in  $184 \times D 1$ . It can be seen also that as the growth-rates at  $26^{\circ}\text{C}$ . of the  $\beta$  series decrease so do the peaks of the curves become blunter until in some strains, e.g.  $111 \times 180$ ,  $124 \times 159$ , and  $150 \times D 1$ , there is little difference between the growth-rates at 22, 26, and  $30^{\circ}\text{C}$ . of each strain, respectively. It must be concluded from these results that both the groups B and C are heterogeneous for factors controlling growth-rate at different temperatures in the diploid. Groups B and C each



TEXT-FIG. 11. For explanation see text.



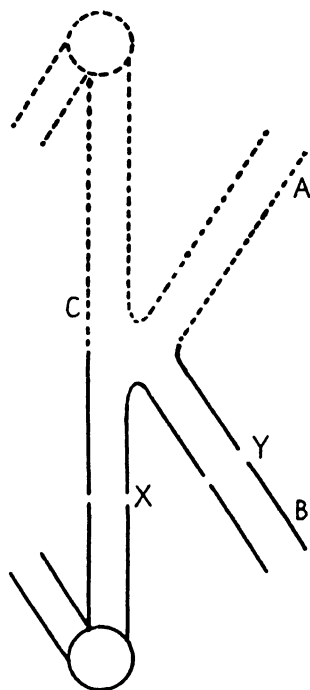
TEXT-FIG. 12. For explanation see text.

contain the factors which produce both the  $\alpha$  and  $\beta$  series in the diploids, and either B or C, or both, contain the factors of the  $\gamma$  series.

In Table II are given the results of pairing random samples of the haploids of groups B and C (i.e. those whose sex was determined, *vide* section 5) with D 1 or D 20. (The diploid D 1  $\times$  D 20 is of the series  $\alpha$  type.) The diploids so produced were measured for growth-rate at different temperatures and were then separated into the three series  $\alpha$ ,  $\beta$ , and  $\gamma$ . While the number of diploids is too small for definite conclusions to be arrived at, it is clear that haploids of group B when paired together give a larger percentage of series  $\alpha$  diploids than do diploids derived from strains of group C. When members of groups B and C are paired together the percentage of series  $\alpha$  diploids is intermediate between the percentages in B  $\times$  B and C  $\times$  C diploids.

## 7. DISCUSSION.

*The influence of the diploid and haploid on one another.* In the preceding paper of this series (5) the theory was tentatively put forward that the paired nuclei of a diploid A  $\times$  B which had diploidized a haploid C entered the latter, divided, and ultimately gave rise to spores, and that at the same time some of the diploid nuclei of opposite sex to those of the haploid paired with the latter forming a new dicaryon combination (C, A), which also gave rise to spores, the two dicaryon combinations being present in the same mycelium. This conclusion was arrived at as a result of comparing the haploid types segregating from the three diploids A  $\times$  B, C  $\times$  A, and C/A  $\times$  B. In the preceding pages experiments of a different nature are described which are also in support of the production of a new dicaryon combination in the diploidized haploid. Two diploids A  $\times$  B and A  $\times$  C were obtained whose growth-rate-temperature curves differed radically from one another (Section III). On diploidizing the haploid C by the diploid A  $\times$  B, the resulting diploid C/A  $\times$  B was found to have a growth-curve indistinguishable from that of A  $\times$  C. It is deduced, therefore, that (A) nuclei of the original diploid (taken either from (A, B) dicaryons or (A) nuclei which had remained unpaired) formed new dicaryons with the (C) nuclei of the haploid. If, as was suggested previously, the (A, B) dicaryons



TEXT-FIG. 13. For explanation see text (p. 242).

were also present in the new diploid, it might be expected that the growth-curve of the  $C/A \times B$  diploid would be intermediate between those of the  $A \times B$  and  $A \times C$  diploids. It has been found, however, that the characters of the (C) nuclei (in this case the haploid was D 17, type *e*, group A) which control the growth reactions in the diplophase are completely dominant to the characters present in any other type of nuclei, so that if dicaryons of type (A, B), (D 1, E 17) be present their influence would not be seen in the growth-rate-temperature curve.

While the influence of the diploid on the haploid has been demonstrated frequently, the converse effect is believed to be new. Using the same haploids as those employed above, it was found that the diploid  $A \times B$ , (D 1  $\times$  E 17), could be affected by contact with a haploid C (D 17) so that its growth-rate was altered. This effect of a haploid on a diploid will be referred to as 'rediploidization'. The growth-rate of the diploid  $A \times B$  falls from 22° C. to a minimum at about 26° C. when it rises a little, reaching a maximum at 30° C. The curve of  $A \times C$ , on the other hand, rises rapidly from 22 to 26° and then more slowly to 30° C. where it attains a maximum. The growth-rate of the rediploidized  $A \times B$ , i.e.  $A \times B/C$ , is about the same as that of  $A \times B$  at 22 and 30° C., respectively, but is much greater at 26° C. where it is intermediate between the rates for  $A \times B$  and  $A \times C$ . It is suggested as a possible explanation that a few (A, C) dicaryons are found in the  $A \times B$  diploid but that their number is not sufficient, despite the already-mentioned complete dominance of type C, to effect a complete alteration in the reactions of the diploid. It has been observed that the difference between  $A \times B$  and  $A \times B/C$  increases with time, that it is greater in colonies derived from young than in those from old inocula, and in colonies derived from inocula incubated at 22° as compared with 26° C. These three factors indicate that the difference increases under conditions favouring active growth, which presumably allow the relative number of (A, C) dicaryons to increase.

*The frequency distributions of the growth-rates of the segregants from eight crosses.* The growth-rates of the parents in the different crosses were very dissimilar. In some crosses both parents were fast growers, in others one was fast and the other intermediate or slow, and again both parents were slow. Segregants of a similar morphological type from different crosses were very similar in growth-rate (Text-fig. 6). The range of growth-rates of the combined segregants of any one cross is almost identical with that from any other cross irrespective of the parental growth-rates. In six out of seven crosses (in the eighth no count was made) one or more of the segregating types occurs with a greater frequency than one or either of the parental types. The exceptional cross (30  $\times$  21) had types *d* and *e* as parents. Types *d* and *e* were found among the segregants in every cross. It is probable, judging from their range of growth-rates, that the strains of at least some of the segregating

types (notably type *d*) are not homogeneous for characters determining growth-rate. The segregants being of the haploid generation, would, in the absence of linkage, be obtained in types of equal frequency. Text-fig. 6, however, shows this not to be the case, peak frequencies being generally found at rates of spread similar to those of the parental strains. It is therefore concluded that linkage between growth-rate factors is present.

These are the chief features of general interest which have appeared from a study of the segregants.

The appearance of certain non-parental types in the  $F_1$  with a frequency greater than that of the parents is capable of several explanations. Firstly, if the fungus were an autopolyploid such a distribution of types would be expected, and a cytological examination was carried out to determine the point. The investigation was made difficult owing to the limited material available (fruit-bodies were difficult to obtain) and the scarcity of diakinesis and metaphase stages. The evidence, however, all pointed to the strain being a diploid with a basic chromosome number 4. This number agrees with that for the majority of the Agaricaceae as determined by Wakayama (9, 10), including the two *Coprinus* species, *C. atramentarius* and *C. micaceus*, which are definitely diploid. Secondly, a frequency greater than 50 per cent. crossing-over, involving two chiasmata per bivalent, the two chromatids crossing-over at one chiasma being different from those taking part in the second. Thirdly, certain chromosome abnormalities would readily account for many of the facts. That chromosome abnormalities would be expected in this fungus might be inferred from a knowledge of its history in relation to the numerous experiments recently carried out on the effects of age and temperature in inducing mutation. The fungus (*vide* Part I of this series) was obtained from an exposed brick forming part of a pyramid in the Fayoum, in Egypt, where it had been exposed to considerable heat for an unknown period of time. These conditions (i.e. age and heat) are similar to those which have been found by numerous workers (2, 3, and 8) to cause mutations (including chromosome aberrations), involving aborted pollen, death in the seedling stage, and poor germination. A low percentage of germination of the spores of *C. sphaerosporus* has not been observed, but the cessation of growth at an early stage of development in a percentage of the sporelings has been evident in all crosses. The following theory has therefore been put forward, though merely as a working hypothesis. Type *e* strains are of the original 'wild type' and contain the full chromosome complement combined with a large percentage of dominant genes. A comparison of the haploid strains of *C. sphaerosporus* with those of *C. lagopus* and *C. macrorhizus* shows the type *e* colonies to be most similar in type and appearance to the homogeneous haploids of the other two species. It will be seen from Text-fig. 6 that the more nearly the parents of each cross approach to the type *e* strain in growth-rate and appearance the smaller



the percentage of dwarf colonies. Type *e*, Text-fig. 11, is completely dominant to all other types so far as growth-rate in the diplophase is concerned. It may here be mentioned in support of the above that a saltant of *C. macrorhizus* induced by X-rays—an agent which not uncommonly gives rise to chromosome abnormalities—in one of the homogeneous haploids, when crossed with a normal type gave rise to a long series of types comparable with those obtained in the crosses recorded here, and also to a large percentage of dwarfs (6). It is further suggested that types other than *e* differ from it in having a number of recessive characters and/or chromosome abnormalities in the shape of translocations and deletions, and that only those pairs of strains which together have the full normal chromosome complement can give rise to perfect fruit-bodies. This would explain the fact that in every cross type *e* was one of the segregating strains and progressive stages of deletion of small parts of chromosomes, inversions, etc. might be expected to give rise to the various degrees of fertility which have been found (4, section 5) in pairing random strains of opposite sex. In an arrangement such as that of Text-fig. 13, for which I am indebted to Dr. C. D. Darlington, where the lower chromosome has the portion XY inverted, it can be seen that the chromatid AYB will be lost in the division owing to the absence of a spindle fibre, and that the chromatid C may break at any point. Assuming such an inversion in several chromosomes it is readily seen how, on inbreeding, a very abnormal chromosome complement can be built up, and it is possible that some such explanation as this may account for the progressive sterility of this fungus during its three years in pure culture. Failing some definite evidence as to the type of chromosome abnormality involved, it is not possible to say whether a high frequency of non-parental types would be expected in the  $F_1$ , but should the loss of a chromosome fragment, accompanied possibly by a translocation such as is suggested in Text-fig. 13, be of frequent occurrence, not many of the  $F_1$  haploids would have a chromosome complement similar to that of either parent, and consequently their morphological and physiological characteristics would be different.

Although numerous series  $\alpha$  diploids have been tested, none have proved fertile, small, undeveloped fruit-bodies alone having been produced. As series  $\alpha$  diploids cannot contain the dominant characters of type *e*, this observation supports the hypothesis put forward above, that only strains which together have the full dominant chromosome complement can mature fruit-bodies. Similarly, although the number of strains tested was less extensive than in the case of series  $\alpha$ , diploids of series  $\beta$  type have never been known to fruit.

In the higher plants factors can influence the development of different parts of a complicated structure, and only rarely do two factors operate entirely in the same field. In the fungi, and especially in the haplophase,

the anatomy is so simple, involving only a branching hypha, that many factors must find expression on the same structure. There is some evidence of correlation between growth-rate and amount of aerial mycelium, a fast-growing strain having in general a more plentiful mycelium than a slow one. It is, therefore, more likely than not that two strains with approximately equal growth-rates will be more alike in appearance than two whose growth-rates are dissimilar. Such a correlation may be due either to linkage or to a manner of growth of this fungus whereby a rapid rate of spread generally involves an abundant aerial mycelium (the latter case does not hold invariably, as type *f*, for example, is a fast grower, but thin). In other words, it may arise either from the linkage of two or more genes or from the majority of genes individually influencing both growth-rate and the amount of aerial mycelium. The suggestion of several factors influencing the development of the same structure in (so far at least as growth-rate is concerned) much the same way leads one to expect the presence of multiple factors whose presence would also account for the high frequencies of non-parental types. Thus, for example, two segregants with similar factors for mycelial development might have different factors for growth-rate which, however, produced the same, or nearly the same, rates of spread. Strains such as these would be grouped together owing to their similar phenotypes. It appears probable that a theory of multiple factors combined with certain chromosome aberrations offers the simplest interpretation of the problem.

*Observations on the diplophase.* All diploids formed from the union of a strain of group A (type *e*) with any other strain, whether of group A, B, or C, are all similar and of series  $\gamma$  type with a maximum growth-rate at 30° C. On the other hand, when the diploid is formed from B  $\times$  B, B  $\times$  C, or C  $\times$  C group crosses it may be of the series  $\alpha$ ,  $\beta$ , or  $\gamma$  types (it is probable that the  $\gamma$  series diploid would be obtained from a B  $\times$  B cross on further diploids being examined). This points to the complete dominance and homogeneousness of type *e* and to the presence of recessives and heterogeneity in each of the groups B and C. It is of interest to note that although at 30° C. the growth-rates of group B strains are high and those of group C low in the B  $\times$  B diploids the  $\alpha$  series types with slow growth-rates at 30° C. are most plentiful, whereas in the C  $\times$  C diploids the  $\beta$  series with fairly high (though very varied) rates predominates.

Among the haploids the 'dip' in the growth-rate of 22° is most marked in group A, and in the diploids it is most in evidence in groups A  $\times$  A, A  $\times$  B, and A  $\times$  C. In the two B  $\times$  C and two C  $\times$  C diploids (Text-fig. 12), of which the growth-rates have been determined at 18° C., it does not show at all, and in the series  $\alpha$  diploids it is not found. The growth-rates at 22° C. of many of the B  $\times$  B, B  $\times$  C, and C  $\times$  C diploids are greater than the corresponding ones in the A  $\times$  A, A  $\times$  B, and A  $\times$  C groups. This

indicates an increase in growth-rate at 22° in the absence of certain group A factors, possibly those responsible for the 'dip'.

A comparison of the results obtained on *Coprinus sphaerosporus* with those of Ashby (1), working in this department on maize, are of interest. In the first place, Ashby showed that the gametes of a homozygous diploid when paired with those of a second homozygous diploid which had a smaller 'efficiency index' than the former gave rise to a diploid indistinguishable from the first as regards efficiency index, or, in other words, that the higher of the two efficiency indices was completely dominant. He also arrived at the conclusion that the indices were inherited as though controlled by a simple Mendelian factor. In the present instance it can be said that gametes (i.e. the haploids) of a certain type (group A, type *c*) always completely dominate the growth-curve of a diplophase irrespective of the characters carried by the other gamete. Apart from this type (series  $\gamma$ , which has the highest growth-rate, and which may perhaps be comparable to the diploid with the greater efficiency index), however, numerous other types of diploid growth-curves were obtained, and it could not be said that one or other type of gamete had characters which were dominant over those of other gametes; in fact, in the two strains D 1 and D 20 where a large number of measurements were made, this was definitely not the case (*vide* Text-fig. 12). In the present experiment two gametes, D 17 (type *e*) and D 1, were paired and the  $F_1$  diploid D 17 + D 1 (series  $\gamma$ ) obtained; gametes from this were crossed among themselves and also back-crossed with D 1, and in the resulting  $F_2$  generation the diploids were of types  $\alpha$ ,  $\beta$ , and  $\gamma$  (all curves in Text-figs. 11 and 12 except those in which D 20 is involved). From this it is concluded that the growth-rate is controlled by more than one pair of character differences.

## 8. SUMMARY.

A haploid diploidized by a diploid was found to have a growth-rate-temperature curve different from that of the latter. This is taken to indicate that a new dicaryon is formed in the haploid, composed of the haploid nucleus and one of the nuclei of the diploid, which is in support of a theory previously advanced from a different set of observations.

It has been found that the growth-rate-temperature curve of a diploid is altered after the diploid has been in contact with a haploid. It is concluded that a haploid colony can rediploidize a diploid under certain circumstances. The difference between the original and rediploidized diploid increases with time, and the rate of change is dependent on the temperature at which the rediploidized cultures are incubated.

Haploid segregants of a cross between two fast-growing strains were examined and were comparable in growth-rate and type to those from other crosses previously made. A comparison of the segregants from eight

simple crosses has been made, from which the following are the chief items of interest. In all crosses numerous types segregated, and the range of growth-rates was very similar in each case. Types *d* and *e* segregated from every cross. In six out of seven crosses one or more of the segregating types had a frequency greater than that of one or either parental type (in some cases a parental type did not reappear in the  $F_1$ ).

The growth-rates at five temperatures were determined in 185 and the sex in 149 segregants from one cross. The segregants (haploids) were found to belong to three types on a basis of their growth-rate-temperature curves.

Diploids derived from these segregants showed three types of growth-rate-temperature curve. These three types had well-defined temperatures for maximal growth-rate of 22, 26, and 30° C., respectively. Some of the strains with a maximum of 22° displayed a second minor maximum at 30° C.

Type *e* haploid strains have characters which are completely dominant in the diplophase to those of any other haploid. It is concluded that more than one pair of factors is connected with growth-rate in the diploid.

Various interpretations of these observations are briefly discussed and a theory based on the presence of multiple factors combined with certain chromosome abnormalities is tentatively advanced as offering a possible solution.

I have great pleasure in thanking Professor F. W. Oliver, F.R.S., for the encouragement and advice he gave me during that part of the work which was carried out in Egypt, and Professor V. H. Blackman, F.R.S., not only for permission to work in this department on various occasions, but also for his advice and very helpful criticism.

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#### DESCRIPTION OF PLATE IV

Illustrating Dr. Dickson's paper on 'Studies in *Coprinus sphaerosporus*. III. The Inheritance of Factors affecting the Growth-rates at Different Temperatures of Certain Strains.'

Each figure represents six-day-old cultures of a single strain grown at (from left to right) 18°, 22°, 26°, 30°, and 34° C. Figs. 1-7 are haploid strains and Fig. 8 is a diploid.

Figs. 1 and 2. Type *e* colonies of group A.

Figs. 3, 4, and 5. Various group B strains. Fig. 3 is a type *d* strain, and it can be seen that while it is very similar in appearance to the type *e* strains of Figs. 1 and 2 (except at 34° C.) its growth-rate at 26 and 30° C. is considerably less.

Figs. 6 and 7. Two strains of group C.

Fig. 8. A C × C diploid, showing irregularity in the production of aerial mycelium at 26 and 30° C.

In all the figures the aerial mycelium is less plentiful at 22° than at 18° or 26° C., though the difference is not well marked in the two examples of group C, Figs. 6 and 7.





# On Certain Features of Floral Construction and Arrangement in the Malvaceae.

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With eighty-one Figures in the Text.

## INTRODUCTION.

AMONG the Malvaceae are included numerous genera, the gynaecium of which exhibits features admittedly difficult to reconcile with the traditional interpretation respecting the character and number of the carpels present. Thus in the section Ureneae the number of styles is twice as many as the number of carpels presumed on the above view to be present, a conception difficult to accept as it stands.

Again, among those members of this section characterized by a gynaecium composed (on the traditional view) of five carpels and having an identical floral formula, some are described as having antesepalous and others antepetalous loculi, while both arrangements are stated to occur in the genus *Pavonia*. But, indeed, the statements in the literature on this point are hopelessly conflicting. If we turn to the descriptions by Baillon (1), Eichler (4), A. Gray (5), and K. Schumann (14) of the five genera *Goethea*, *Malachra*, *Malvaviscus*, *Urena*, and *Pavonia* we find that only in respect of one of these genera, viz. *Goethea*, are these observers in agreement in their statements regarding the position of the loculi. That there is some special difficulty in determining this relation in these types would appear from the fact that in his most recent article on the Malvaceae, Schumann at one point describes *Malachra* as having carpels (and therefore loculi) epipetalous (loc. cit., p. 45) and in the same account (p. 32) he includes this genus in the list of those cited as having carpels episepalous. It may be added that Bentham and Hooker (2) give the position of the loculi in all these types with a ?.

Yet again, in the Malopeae, in which the number of carpels may be very large, they are described as being grouped in five fields and so arranged as to form a number of vertical rows, those of each new tier being superposed upon those of the preceding one. Moreover, the five groups of carpels are represented as standing in line with the petals, so that here, as



in some of the Ureneae (see above), all the whorls after the calyx, i.e. those of corolla, androecium and gynaecium are held to be superposed.

Finally, in many genera the style column is 'gynobasic'. Even so the problems presented by the malvaceous flower are not exhausted. For although it is well known that each staminal filament bears only half an anther this anomaly still requires elucidation.

In the account which follows it is shown that the clue to the aberrant features occurring in the androecium and gynaecium is to be found in the vascular ground-plan, and that on the theory of Carpel Polymorphism those characterizing the gynaecium no longer constitute anomalies but fall into line. Further, that there is no real transgression of the principle of alternating whorls *if the unit taken into consideration is not the floral member but the vascular unit delimited at any one time by a single process.* [For an earlier reference to this conception of the fundamental basis of alternating whorls see (12), and for a fuller account (13).]

The general scheme of the relations of the several whorls is most easily appreciated in the Hibisceae, and illustrative types belonging to this section will therefore be considered first.

#### *Hibisceae* (Figs. 1-18, 25-8).

Style column terminal. Style branches equal in number to the loculi, both being antesepalous in *Hibiscus*, but when five in number in *Gossypium* they are antepetalous.

The genera examined included *Hibiscus* with an isomalous gynaecium, *Gossypium* having frequently, and *Julostylis* and *Dicellostylis* having constantly, an oligomalous gynaecium.

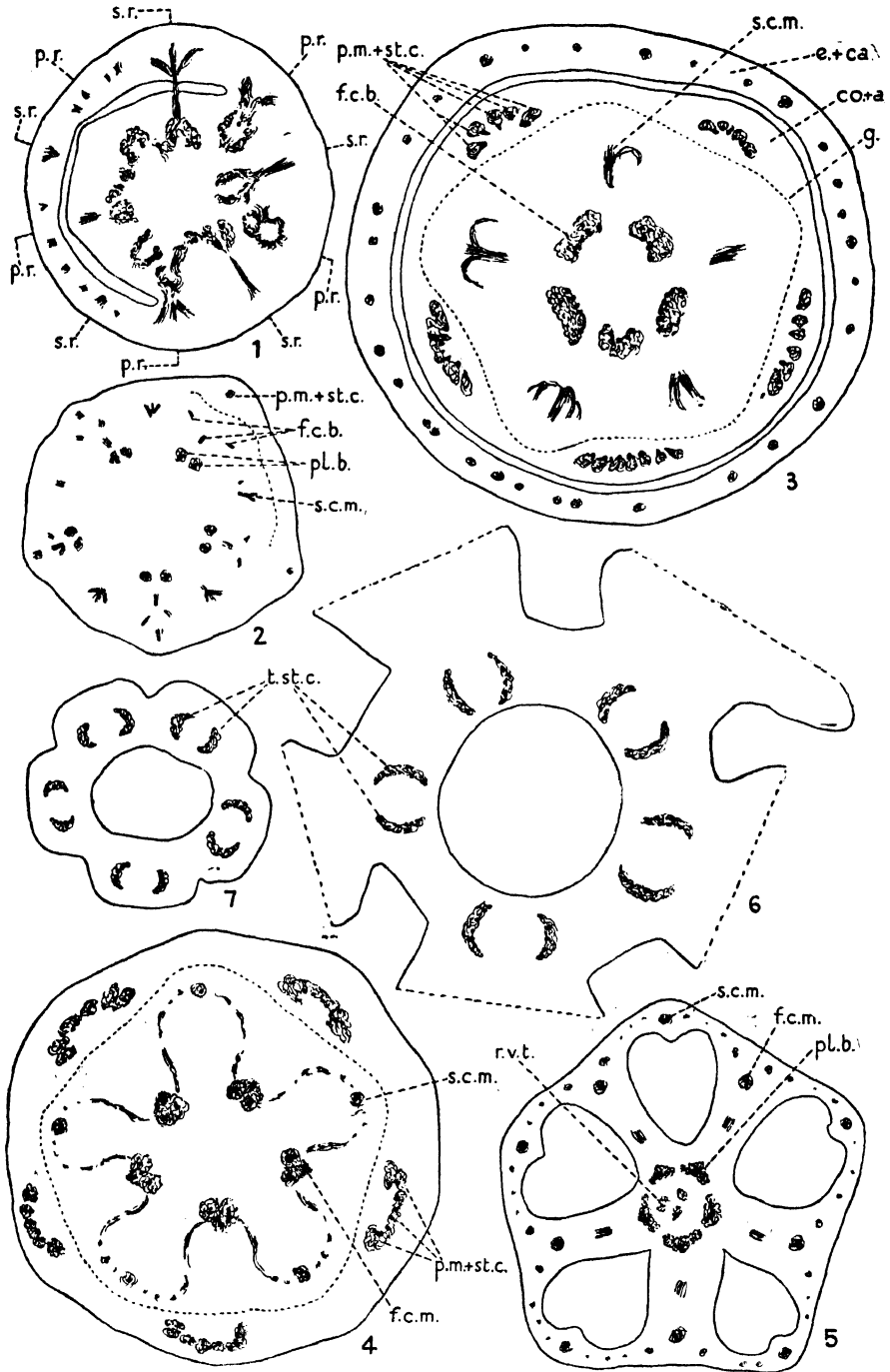
*Hibiscus* (Figs. 1-18). Observations were made on *H. Rosa-sinensis* L. f., *H. syriacus* L., *H. Trionum* L., and an unidentified species from S.E. Africa, all with epicalyx. The arrangement of the vascular tissue in the region immediately below the flower is irregular, for the number of bracts in the epicalyx in most cases bears no symmetrical relation to the ten construction radii of the flower. After the emergence of the epicalyx bundles sepal midrib bundles turn outwards on one set of radii, sepal commissural laterals conjoined with a petal-stamen trunk cord on each of the alternate radii.<sup>1</sup> The succeeding whorl, that of the five sterile carpels is, therefore, naturally formed on the sepal radii.<sup>2</sup> The whole of the

<sup>1</sup> The distance from the residual vascular cylinder traversed by the petal-stamen trunk cords before they become resolved into their components varies in different species. In some these cords pass out unresolved into the corolla-androecium tube, in others they break up on their way to the periphery of the axis into a petal midrib bundle flanked on each side by a cord which supplies one-half of the superposed group of stamens.

<sup>2</sup> For each petal and superposed group of stamens being supplied by a common trunk cord, corolla and androecium behave as one whorl as regards the operation of the principle of alternation (see (12) and (13)).

residual vascular tissue on these radii is utilized to provide the sterile carpel midribs which branch as they approach the periphery and turn upwards. Of the residual vascular tissue lying on each of the alternate radii only a portion turns outwards to furnish a fertile carpel midrib with its lateral branch system. The residual elements left in the centre, except for a few here and there which turn inwards into the central parenchyma and end blindly, give rise to the placental strands. The central parenchyma itself shortly afterwards ceases, giving place to conducting tissue which lines the five channels leading into the loculi and entirely fills the common stylar canal. As the loculi narrow above to mere slits the fertile carpel midribs split in half. The two resulting bundles turn inwards and pick up the placental strand on the corresponding side. This change in direction is accompanied by a change in the outline of the ovary, which now shows a marked indentation in the mid-line of the fertile carpels. The sterile carpel midribs are also prolonged upwards, while their laterals either cease or anastomose with the fertile carpel bundles. Hence, as the ovary passes into the terminal five-fluted style column three bundles are to be seen in a cross-section in each of the arcs between the grooves. Each group of three bundles is composed of a sterile carpel midrib in the middle and on each side half the vascular system of the neighbouring fertile member on that side. Each arc thus represents  $\frac{1}{2} + \frac{1}{2}$  carpels. Shortly before the style column separates into its five stigmatic branches the sterile carpel midribs come to an end. Each of the five branches consequently shows only the two bundles derived from the two neighbouring fertile carpels. These latter carpels have the characteristic form of the semi-solid type. Each bears two rows of ovules; one row lying in each of the neighbouring loculi. The sterile carpels from their shape and venation might be classed either as much contracted valve, or as rather expanded solid carpels, it being impossible in the case of such intermediate forms to draw a sharp line between the two types when sterile.

*Gossypium barbadense* L. (Figs. 25-7), *G. herbaceum* L. The flower of *Gossypium* is slightly perigynous. It differs from the preceding types in the inconstancy of the number of carpels (5-2) and, more profoundly, as regards the relations of the two carpel whorls. At the flower base the vascular cylinder expands greatly and breaks up in such a way that a transverse section shows a large number of vascular strands scattered throughout. Those situated peripherally become organized into the bundles for epicalyx, calyx, corolla, and androecium; those lying in a belt near the centre furnish the circle of strands running upwards in the wall of the ovary; those still more centrally placed become condensed into the placental strands. No definite bundle occurs in the ovary wall in line with the loculi. Each mid-line is marked instead by a longitudinal rib of non-vascular tissue which projects in the basal region of the ovary from the



FIGS. 1-7. Hibisceae. Figs. 1 and 2. *Hibiscus Trionum* L. Fig. 1. Flower base after partial exertion of the epicalyx-calyx ring showing stages in the formation of the trunk cords for part of the epicalyx system and the sepal midribs on the one set of radii, for part of the epicalyx system,

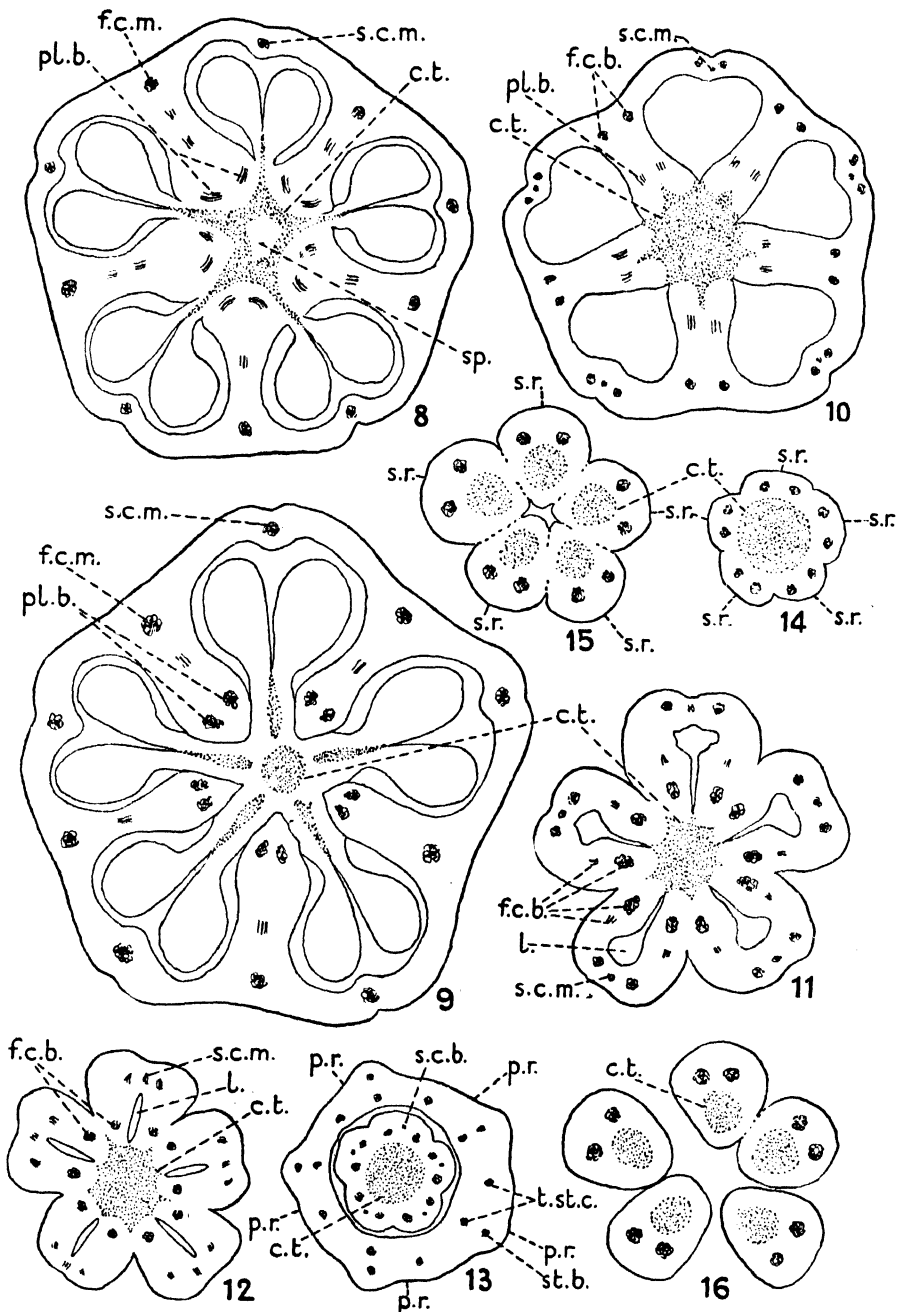
outer wall into the loculus. In these ribs a median sheet of cells shortly becomes differentiated, extending radially from the outer epidermis to the inner face bounding the loculus. These cells, arranged in regular radial rows, have all the appearance of being the meeting layers of two potential surfaces and recall the actual channel to be seen on the corresponding radii in the ovary of the tulip. I have suggested that this channel arises through the contraction of the intervening sterile carpel from a sector of the ovary wall to a mere column of tissue enclosing the midrib bundle, thus permitting part of the radial face of the fertile carpel on each side to acquire a free surface (7) (p. 163, Fig. 78). A similar explanation may be offered of the above-mentioned layers in *Gossypium*. But in this latter genus a further modification of the original ground-plan has evidently taken place, so that the rôles of the two carpel whorls have become reversed. For here the antesepalous whorl constitutes almost the whole of the ovary wall and also forms the 'septa'. These members are of typical semi-solid form. They

sepal commissural laterals and corolla-androecium system on the other set of radii. Fig. 2. The same after exertion of the epicalyx-calyx ring and partial delimitation of the corolla-androecium ring. In line with the petal-stamen trunk cords the branching main bundle and twin placental strands of the fertile carpels; on the alternate radii the midribs of the sterile carpels, some of which have given rise to a pair of laterals. [In both Figs. 1 and 2 one side of the flower is further developed than the other.] Figs. 3-7. *H. Rosa-sinensis* L. Fig. 3. Flower base. On the outside the exerted epicalyx-calyx ring which has been left in position; the bract and sepal bundles are not yet differentiated from one another. Within this ring the corolla-androecium tube and gynaecium delimited but not yet disjoined. Towards the periphery of the gynaeceum on the sepal radii the sterile carpel midribs in process of turning outwards and branching. Nearer the centre on the petal radii the vascular units supplying the fertile carpels delimited but not yet organized. Fig. 4. The corolla-androecium ring and gynaeceum as in Fig. 3. The fertile carpel bundles are now in process of becoming organized. Fig. 5. The gynaeceum after the appearance of the loculi. The fertile carpel vascular system is now fully organized. In the centre groups of undifferentiated vascular elements. [For the sake of simplicity the ovules have been omitted.] Fig. 6. The corolla-androecium tube after the petals, of which the distal portion has been cut away, have been detached. Fig. 7. The androecium tube with its five pairs of vascular cords. All from transverse sections taken when in series from below upwards and magnified equally.

As the abbreviations used in explanation of the text-figures are the same throughout, the complete list is given here in order to avoid repetition:

*a* androecium  
*apgo* antepetalous group of ovaries  
*asgo* antesepalous group of ovaries  
*c* canal  
*ca* calyx  
*ce* cut edge of petal  
*cp* central parenchyma  
*co* corolla  
*ct* conducting tissue  
*ds* dorsal radial split  
*e* epicalyx  
*eb* epicalyx bundle  
*fb* funicle bundle  
*fc b* fertile carpel bundle  
*fc m* fertile carpel midrib  
*g* gynaeceum  
*ip* inner phalange of stamens  
*is* interstitial radial split  
*l* loculus  
*o* ovary  
*op* outer phalange of stamens  
*ov* ovule

*pl b* placental bundle  
*pm* petal midrib  
*pr* petal radius  
*ps* potential surfaces  
*rvt* residual vascular tissue  
*sc b* sterile carpel bundle  
*sc l* sepal commissural laterals  
*sc m* sterile carpel midrib  
*sm* sepal midrib  
*sp* space  
*sr* sepal radius  
*st b* stamen bundle  
*st c* stamen cord  
*stip* stigmatic papillae  
*sty c* style column  
*sty f* style filament  
*tst c* twin stamen cords  
*ucc* unresolved carpel cords  
*uma* unit member of androecium  
*uscl* unresolved sepal commissural laterals  
*vs* ventral radial split



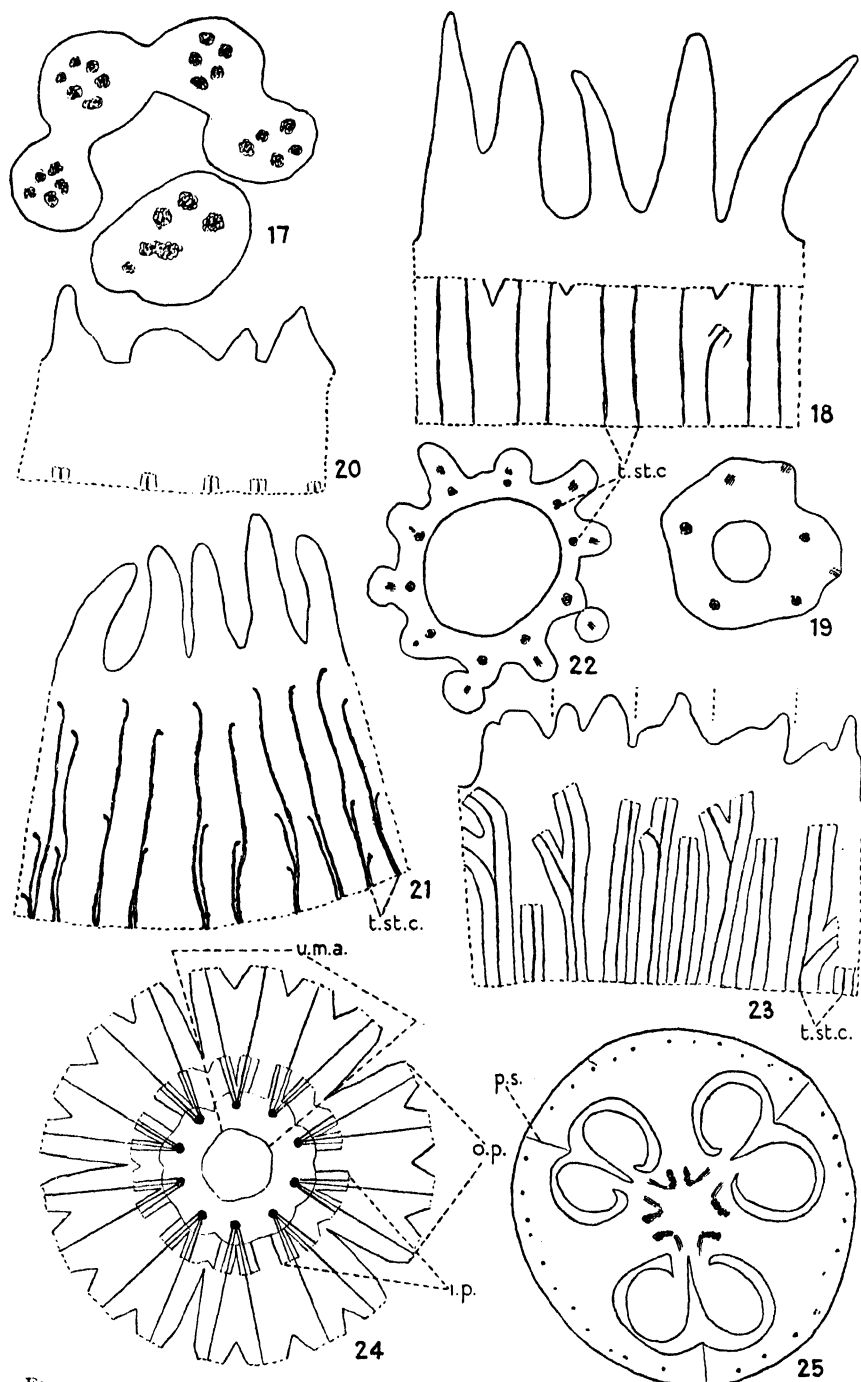
FIGS. 8-16. *Hibisceae. Hibiscus Rosa-sinensis* L. Fig. 8. The gynaecium after the central parenchyma has given place to a cavity bounded by conducting tissue which connects with the loculi. Fig. 9. The same after the central cavity has become filled with conducting tissue. Fig. 10. The same shortly before the loculi begin to narrow above, showing extensions of the large central core of conducting tissue in the mid-line of the fertile carpels as they begin to split radially from

are furnished with an abundant vascular system and they have taken on the fertile function. The antepetalous carpels, on the other hand, having become functionless have all but disappeared, being represented only by the very short basal region of the non-vascular rib mentioned above, in which appears almost at once the radial sheet of cells indicating the junction line of the fertile carpels which became continuous as the sterile members came to an end. This reversal of the relations, both morphological and functional, of the two carpal whorls explains the otherwise puzzling fact that in completely isomerous flowers of *Gossypium* the loculi are antepetalous, whereas in *Hibiscus* the carpels which are sterile, and hence also the loculi, are anteseptalous.

A similar interchange of the carpellary functions, as I have previously pointed out (8, p. 302, and Figs. 2-4), is to be seen in the Resedaceae. For example, *Reseda luteola*, *R. odorata* and *R. lutea* all have an ovary of six carpels with a similar floral ground-plan, yet in the first-named species it is the anterior and two postero-lateral members which are solid and sterile, whereas in the other two species these are the members which are semi-solid and fertile. Again, in the relation of all three species to *Astrocarpus scsamooides* we have a still closer parallel to that between *Hibiscus* and *Gossypium*, for in *Astrocarpus* there are no sterile members; all the six carpels present are semi-solid and fertile. Each encloses a single ovule which is borne on the midrib. These carpels afford, in fact, a perfect example of the semi-solid or pseudo-valve class, here, in an apocarpous gynaeceum, whereas they are more usually found in the syncarpous condition. This (on the traditional monocarpellary view) exceptional form of placentation has long been known. It is mentioned by Payer (6, p. 197) and figured by Strasburger (15, p. 58 and Pl. 8, Fig. 92). Payer, indeed, lays emphasis on the very singular structure of these carpels which, he adds, has at all times exercised the sagacity of botanists. Despite the plain facts orthodox botanical opinion has been content to leave the matter thus and to continue to recognize only the traditional valve type of carpel.

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within outwards. [For the sake of simplicity the ovules have been omitted.] On the sepal radii a weak sterile carpal midrib flanked by strong laterals. On the petal radii twin strands representing a fertile carpal midrib. Fig. 11. The same after the loculi have begun to narrow. The fertile carpels have begun to split radially from without inwards and the twin strands of their midribs to turn inwards. Fig. 12. The same immediately before the loculi close. The twin strands of each fertile carpal midrib have picked up in their inward course the corresponding twin placental strands. Fig. 13. The androecium tube and basal region of the five-fluted style column, the five arcs in the outline of the column being centred in line with the loculi. In each of the five sectors corresponding with one of the arcs, three vascular bundles representing the weak midrib of a sterile carpal and half the vascular system of the neighbouring fertile carpal on each side more strongly developed. Fig. 14. Middle region of the style column. The five weak sterile carpal midribs having come to an end only the two strong bundles of the fertile carpels seen in Fig. 13 are present in each sector. Fig. 15. Upper region of the same after the break-up of the central core of conducting tissue seen in Fig. 14 into five separate tracts standing in line with the sterile carpels. The vascular bundles as in Fig. 14. The five filaments are becoming delimited and have already separated along their inner face. Fig. 16. The five filaments now almost completely disjoined. All from transverse sections taken when in series from below upwards and magnified equally.



FIGS. 17-25. Fig. 17. *Hibiscus Rosa-sinensis* v. *Colesii* hort. Transverse section of the staminal tube at the level at which it begins to split into the five terminal sterile structures, each of

*Dicellostyles axillaries* Benth., *Julostylis angustifolia* Thw. (Fig. 28).  $G_2+2$ . In both these genera, which have a typical pentamerous calyx, corolla, and androecium, the central cylinder expands at the flower base. Strands turning outwards in an irregular pattern become organized into the epicalyx system. Thenceforward the organization of the bundles for the successive whorls proceeds centripetally. Five cords take up a position on the sepal radii and another five come to stand on the petal radii. From the inner face of most or all of these ten cords strands are detached which are incorporated into the outer wall of the ovary, after some have in turn given off towards the centre other strands which become organized into two placental bundles. The ten above-mentioned cords continue upwards and become the sepal midribs and the petal-stamen cords. Of the detached strands which enter the ovary wall five usually continue as the midrib bundles of the two sterile and the two fertile carpels, two functioning as a single midrib for one of the fertile members. The fact that besides these five strands additional ones are usually present, derived from some or all of the other original ten cords, and hence standing on different radii, indicates that these additional bundles are proper to some or all of the

which stands over the vertical rows of fertile stamens constituting a single antepetalous group. In each sector corresponding to one of these structures a cluster of vascular strands derived from the twin cords seen at a lower level which serve one antepetalous group of fertile stamens. Fig. 18. *Hibiscus Rosa-sinensis* v. *Archeri*. Apical region of the staminal tube split open and laid flat showing five terminal sterile teeth standing over the five pairs of vascular cords corresponding with the five clusters of strands seen in Fig. 17. The filaments of the topmost fertile stamens have been cut away immediately above the level at which they spring from the tube; their bases, in some cases not yet free laterally, give the appearance of a truncate lining to the tube (indicated by the interrupted line across the middle of the figure). Figs. 19, 20. *Lavonia spinifex* Cav. Fig. 19. Transverse section taken near the summit of the staminal tube. The vascular complement for three of the antepetalous groups (below and on the left) has become reduced to a single bundle. That for the two remaining stamen groups (above and on the right) still consists of two bundles, one of which in each case is about to enter the last fertile member of the corresponding vertical row. Fig. 20. Apical region of the staminal tube as in Fig. 18. The vascular complement of each antepetalous group of stamens has become reduced to a single bundle which has turned out into the last (topmost) fertile filament of the corresponding vertical row. These filaments have been cut away immediately above the level of origin in order better to expose the alinement of the vascular bundles and the terminal sterile prolongations of the tube which are in the form of three single lobes and one large lobe representing two fused together. Fig. 21. *Malvaviscus mollis* DC. Apical region of the staminal tube as in Fig. 18 showing five terminal sterile teeth in line with the five pairs of vascular cords which supply the vertical rows of the five antepetalous groups of fertile stamens. Figs. 22, 23. *Goethea strictiflora* Hook. Fig. 22. Transverse section of the staminal tube showing the five pairs of staminal cords from which some of the bundles supplying the vertical rows composing each antepetalous group of stamens are becoming detached. Fig. 23. Apical region of the staminal tube as in Fig. 18 showing an irregularly lobed rim owing to secondary lobing of the five primary lobes which stand over each of the five pairs of vascular cords supplying the vertical rows of fertile stamens composing each antepetalous group. [This alinement is exposed by the interrupted vertical lines above the figure which represent the antepetalous radii.] Fig. 24. *Sidalcea malvaeflora* A. Gray. Apical region of the staminal tube as viewed from above in a preparation which has been rendered transparent (semi-diagrammatic). In a ring round the central cavity the five pairs of vascular cords from which are detached the bundles for all the filaments which cohere in phalanges forming an outer and an inner ring. (For the nature of the relation of these phalanges to the true antepetalous groups see p. 255.) Fig. 25. *Gossypium barbadense* L. Transverse section of a trimerous ovary. Near the periphery alternating with the loculi the numerous vascular bundles of three semi-solid fertile carpels, nearer the centre on the same radii the twin placental strands of these carpels. Projecting into each loculus in the mid-line a rib or ridge of tissue interpreted as formed of the persistent non-vascular remnant of the sterile carpel present in other genera. In the ovary wall in the mid-line of this ridge is a radial band of cells suggestive of potential surfaces.



three outer and three inner carpels which no longer take shape, and that in consequence of the suppression of these members they have become incorporated 'where they stand' into the structure of those members which have persisted. This incorporation process accounts for the asymmetry in the vascular system of the ovary which is not infrequently seen in types in which pentamerous whorls of sepals, petals, and stamens are followed by dimerous whorls of carpels, especially when the flower is partly or wholly syngonous as, e.g., in *Lobelia* and *Campanula*.

It remains to treat in somewhat further detail certain unique features of the androecium regarding which there is still lack of unanimity of opinion. It must be emphasized in the first place that in no case is a vestige of the antesepalous whorl present. The whole number of fertile stamens undoubtedly correspond to a single whorl of five antepetalous members, the primordia of which have undergone a primary collateral (radial) splitting and have thus given rise to 10-∞ half stamens<sup>1</sup> usually arranged in paired vertical series. This relationship can be particularly easily observed in *Anoda hastata*, where the filaments are plainly massed in five antepetalous groups. It is, moreover, entirely confirmed by the vascular ground-plan, for the vascular system of the twin vertical series constituting one antepetalous group is derived from the same delimited portion of the central vascular system as the midrib of the corresponding petal, hence the superposition upon the petals of the one staminal whorl which is developed. In general the staminal tube, if it is not crowned by the last-formed fertile filaments, has a truncate apex, but among the *Hibisceae* and *Ureneae* it is frequently surmounted by distinct teeth, generally five, sometimes more.<sup>2</sup> These teeth have been, and still are, regarded as the persisting sterile remnants of the missing antesepalous whorl. Duchartre (3, pp. 136, 137), who first drew attention to these structures and suggested their significance, states that he had been able to convince himself that they alternated with the petals. Later, Asa Gray (5, p. 44), observing in the genus *Sidalcea* that the staminal tube ended in an outer and an inner set of phalanges alternating, as it appeared, with one another, found in this arrangement further evidence bearing out Duchartre's view. So simple and sufficient does this explanation appear that it seems to have become generally accepted on the latter observer's authority without further confirmation, although some doubt as to its validity is raised by certain statements and figures by Payer (6), which are, however, in part contradictory. For this latter writer describes these terminal teeth in *Malvaviscus arborea* as alternating with the petals; nevertheless, in the illustrations of this species they are clearly shown opposite the petals (loc. cit., Pl. 6, Fig. 4). On the other hand, in *Pavonia hastata* they are

<sup>1</sup> i.e. stamens with monotheical anthers.

<sup>2</sup> Owing to lobing of the primary teeth.

stated to stand opposite the petals, but in this case the accompanying illustration (loc. cit., Pl. 7, Fig. 9) gives no help on this point. Apart from these somewhat unsatisfactory findings, the question of position appears to have received no further attention.

Examination of species of *Hibiscus* (Figs. 17, 18), *Julostylis*, *Pavonia* (Figs. 19, 20), *Malvaviscus* (Fig. 21), and *Goethea* (Figs. 22, 23) in which these teeth are well developed shows, however, that they are in fact the terminations of the five fertile antepetalous members, and not of a supposed aborted sterile antesepalous whorl of the androecium, of which, indeed, there is absolutely no trace. This relation, not always easily ascertainable by inspection owing to torsion of the tube,<sup>1</sup> is at once established if the position of the teeth is considered in regard to the staminal vascular cords, for the teeth are then seen to be prolongations of those sectors of the tube which include the twin cords constituting the vascular complement of one complete antepetalous group of stamens. This is particularly easily made out in certain cultivated varieties of *Hibiscis Rosa-sinensis*. As a rule the sterile teeth in question are non-vascular, but in *var. Archeri hort.* and *var. Colesii hort.* of the above species the originally separate twin cords standing in line with each petal come together at the top of the tube, so that ultimately there is but a single cord or cluster of strands on each petal radius. Furthermore, the small teeth of the type here develop into petaloid structures, often of large size, into which these cords are continued, sometimes giving rise to a well-developed branch system. This prolongation of the vascular bundles establishes beyond all question the true alinement of these sterile structures with each of the five antepetalous members.

We now come to the appearance in *Sidalcea* that the fertile stamens are arranged as, and represent, two alternating whorls, one antesepalous and one antepetalous, an appearance which is also found on examination to be illusory. The filaments, as described by A. Gray, are here bunched together at the top of the tube, where they are arranged in an outer and an inner set of phalanges. The number of filaments in each outer phalange varies with the species, but in the specimens examined four were found in *Malvaeflora* and six in *Candida*. The number composing each inner phalange in both these species appeared to be generally four, arranged in two pairs. Now it is to be noted that the vascular system in the tube is exactly similar in origin and ground-plan to that found in every other malvaceous genus, twin cords representing together the vascular complement of one antepetalous staminal member being present in each antepetalous sector of the tube. Towards the summit of the tube these cords break up, each dividing tangentially so that each antepetalous sector now shows two outer and two inner bundles. The two outer bundles in each sector give

<sup>1</sup> Even in the freshly opened flower of some species the surface of the tube has the twisted appearance of a rope.

rise by further division(s) to as many strands as there are filaments in the corresponding outer phalange, the strands originating in one antepetalous sector supplying one phalange, and one strand entering each filament. Similarly the pairs of filaments composing the inner phalanges are all supplied by strands derived by later forking from the two inner bundles, in each antepetalous sector produced by the original tangential division. The position is then that the whole number of stamens, here as in other Malvaceae, receive their vascular supply from the five pairs of cords in the five antepetalous sectors. It follows that the whole number represent the antepetalous whorl only.

Grouping into phalanges, it must be emphasized, is a secondary development, the result of cohesion, which in *Sidalcea* is by no means always regular or of uniform extent. The phalange may or may not be composed of the components of, and hence be the equivalent of *one* of the five members of the whorl. It is not so, as we know, in the *Fumarioideae*. In the present genus, where an outer and an inner ring are present, both conditions are met with. Each outer phalange is composed of some, but not all, of the filaments representing one member of the whorl, while each inner phalange consists of the remainder of the filaments belonging to two neighbouring members. It is this difference in grouping in the outer and the inner set which led Gray to interpret the inner ring as being the antesealous whorl, though he makes no comment on the difficulty involved in supposing that the antesealous, and therefore properly outer, whorl can be completely enclosed by the properly inner antepetalous whorl. That this is an erroneous interpretation is clear from the facts detailed above, from which it is evident, as there stated, that here as in the rest of the Malvaceae there is no trace of the missing antesealous whorl.

One further point of analogy between the androecium of the Malvaceae and the *Fumarioideae* is worthy of remark. As I have pointed out in discussing the ground-plan of *Corydalis* (10, p. 211), if the vascular complement proper to a floral member is halved *before* the bundle is fully organized and delimited from the vascular elements appropriated to other whorls, and if the two halves do not reunite, then the corresponding floral member will be replaced by two separate half members. Subsequent further halving or branching of these half strands *after* they have become isolated will result, not in further fractionization of the members which they serve, for the determinate phase has now been reached, but in the multiplication of the product of the original bipartition. In the *Fumarioideae* there is no subsequent division of the bundles after the first halving, hence only one pair of half stamens replaces the whole member present in the allied *Hypicoideae*. In the Malvaceae repeated division usually occurs after the first halving, hence a large number of half stamens are produced in place of a single whole member. This underlying cause of the formation of the half stamen holds

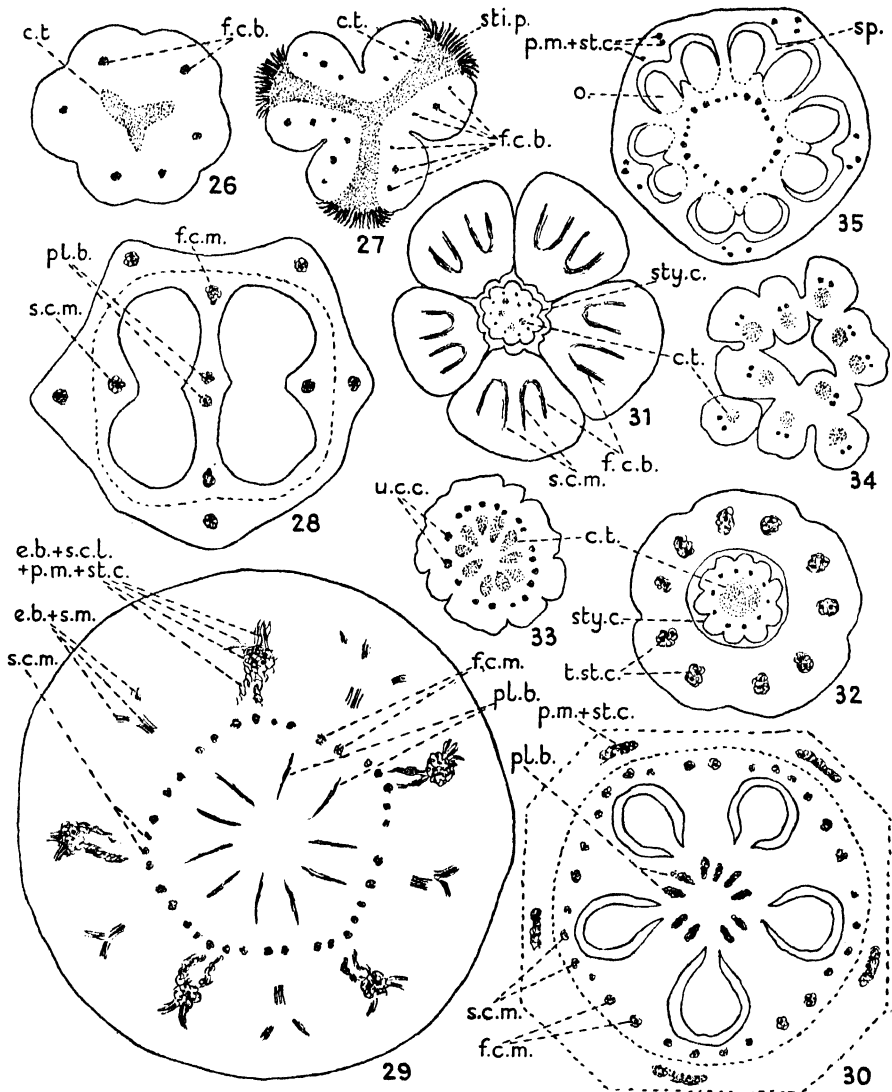
good throughout the family, hence it will be unnecessary to treat the androecium in detail in the following sections.

*Ureneae* (Figs. 19-23, 29-34, 36-57).

Style column terminal or springing from the inner face of the carpels. Style branches twice as many as the loculi. The loculi of certain genera are antesepalous according to some writers and antepetalous according to others (see above, p. 247).

Species investigated in detail: *Pavonia multiflora* A. St. Hil. and *P. spinifex* Cav., *Malvaviscus mollis* DC., *Urena lobata* L., *Goethea strictiflora* Hook.

In *Pavonia multiflora* (Figs. 36-45) epicalyx and calyx are exserted as one structure, the bracts (10 in the specimen examined) and sepals becoming disjoined later. After the emergence of the vascular bundles serving these two whorls five large cords turn out from the central cylinder on the petal radii. These cords leave behind in their outward course twin strands constituting the two halves of a sterile carpel midrib and continue outwards as the petal-stamen trunk cords. Shortly afterwards two similar strands organized independently from elements in the residual cylinder and similarly representing the two halves of the midrib of one of the alternate fertile carpels, take up a corresponding position on each sepal radius. The fact that the carpel midribs on the petal radii are carried outwards with the petal-stamen cords while the corresponding midribs on the sepal radii are organized independently, and therefore later, accounts for the fact that the outer carpel whorl, and hence also the loculi, are antepetalous. The sterile carpel strands are but poorly differentiated and their later course is difficult to trace, but appearances suggest that the twin strands of one midrib diverge before the loculus closes and each turning inwards round the outer angle joins up with the near-by strand, this adjacent strand being one of the pair composing the neighbouring fertile carpel midrib, which has similarly turned inwards, picking up the corresponding placental strand in its course. Thus five pairs of bundles pass into the style column (here terminal as in *Hibiscus*) opposite the sepals. Alternating with these pairs of bundles at this level are five tracts of conducting tissue which stand in line with the sterile carpels and the petals. These separate tracts shortly come into contact, forming a single tract with a five-lobed outline. At a higher level the original five tracts again become distinct while a new, inner set of five now make their appearance on the alternate radii. At the same time the column, which up to this level has in cross-section an even circular outline, becomes ten-fluted, the ten convex arcs being centred in line with the above ten tracts. The ten bundles which entered the base of the column, each of which, in view of subsequent events and of the relations observed in other members of the *Ureneae* (see also above), may be supposed to represent

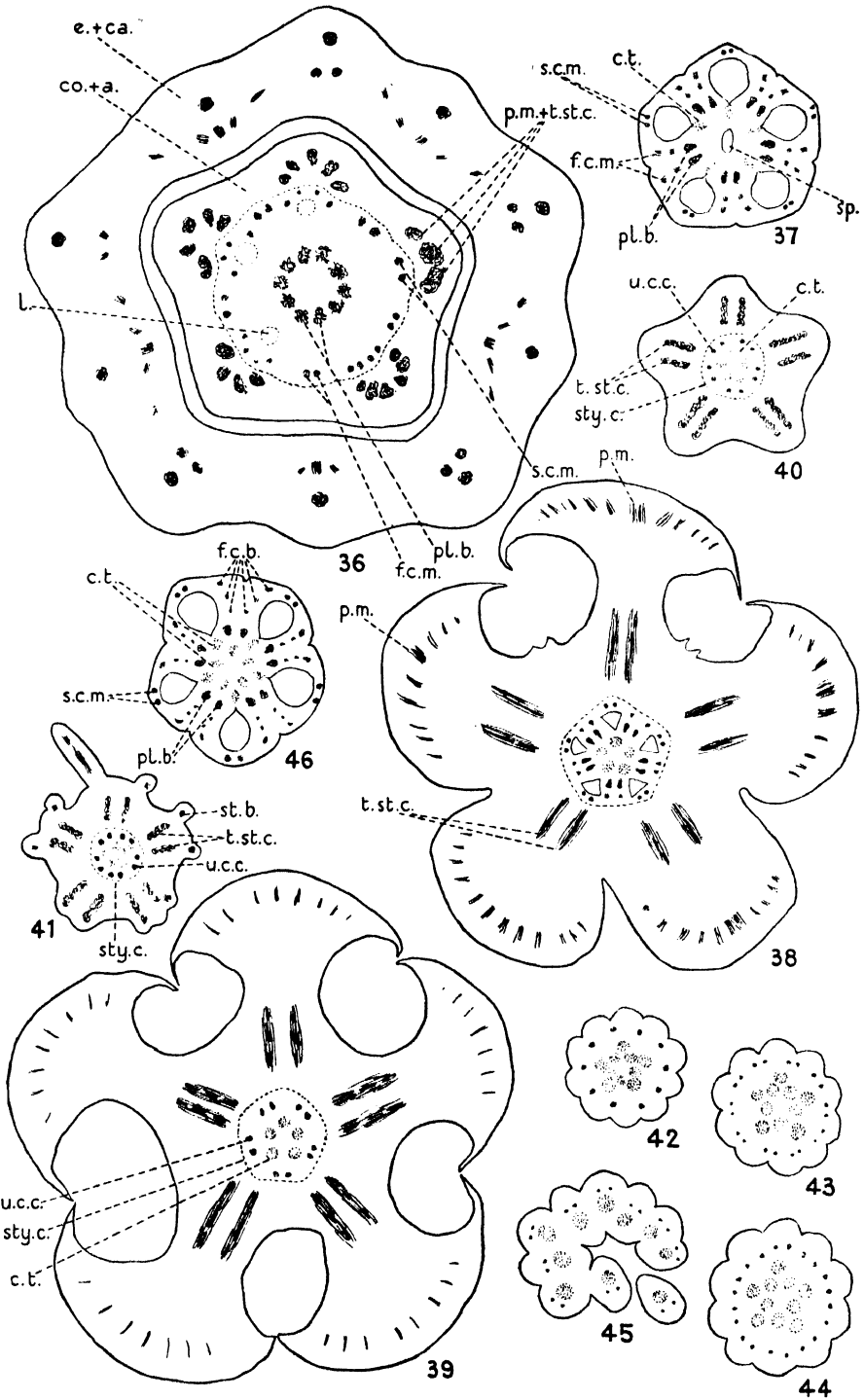


FIGS. 26-35. Figs. 26-8. Hibisceae. Figs. 26 and 27. *Gossypium barbadense* L. Fig. 26. Basal region of the style column showing three pairs of vascular bundles corresponding with the three component fertile carpels. In the centre a core of conducting tissue, three-rayed, the rays standing over the loculi. Fig. 27. Apical region of the same after the rays of conducting tissue have extended to the dorsal surface. The vascular bundles seen in Fig. 26 have broken up into smaller veins. Fig. 28. *Julostylis angustifolia* Thw. The gynaecium surrounded by the corolla-androecium tube, from which it is delimited but not yet disjoined. In the surrounding ring the five petal-stamen trunk cords. Within this ring the midribs of the two sterile carpels and the midribs and placental bundles of the two fertile members. Figs. 29-34. Ureneae. *Malvaviscus mollis* DC. Fig. 29. Flower base. Towards the periphery vascular cords in process of becoming organized into the bundles for epicalyx, calyx, corolla, and androecium. Nearer the centre a ring of strands which are becoming organized into the pairs of bundles representing the midribs, and some secondary veins of the five sterile and the five fertile carpels. Within this ring five pairs of strands cut longitudinally, which become the placental bundles of the fertile members. Fig. 30. The same after removal of the epicalyx-calyx ring. Through torsion of the corolla the petal-stamen vascular cords

half the vascular system of a fertile carpel conjoined with half the vascular system of a sterile carpel, stand in line with the ten grooves. Each of these bundles shortly divides radially in two. The two halves diverge somewhat, so that presently in place of a single bundle in line with each groove two strands come to stand before each arc. This halving of the original bundles brings about (presumably) the separation of the two components which went to their make-up; consequently the two strands now standing before each arc will represent the two halves of a single carpel midrib. Hence when the column presently separates at the grooves into ten stigmatic filaments, each with its twin strands and tract of conducting tissue, these ten filaments constitute the termination respectively of the five sterile and the five fertile carpels. *The explanation of the number of styles in the Ureneae is not, therefore, that there are as many again as there are carpels; but that the unusual condition is found here in which not only is each fertile as well as each sterile carpel prolonged to form a style, but the individual style filaments are all also distinct.* Thus Pavonia (and the statement applies equally to the other genera in this section) affords particularly pretty proof, proof to the open mind beyond question, of the theory of Carpel Polymorphism. The case, however, is not unique. Although the formation of *separate* styles by both the sterile and the fertile carpels of the same ovary is rare, it occurs in even more striking fashion (because the two kinds are dissimilar) in the section Paepalanthoideae of the Eriocaulaceae (see (7), pp. 157, 158) and also occasionally in Eschscholzia (see (7), pp. 133-135, also (9), p. 617).

It is interesting to note that Payer, observing that the Pavonia gynaeceum in its earliest stage shows ten primordia, likened its appearance to that of a pistil of ten carpels, each alternate one of which fails to develop a loculus (6). But it would seem that he meant this comparison to be taken rather as a description conveniently indicating the appearance than as an

no longer stand strictly in line with the loculi. Fig. 31. The five-lobed crown of the ovary enclosing the base of the 'gynobasic' style column. In the centre of each of the five lobes the twin bundles of the sterile carpel midrib are seen turning inwards over the closed loculus on their way to enter the style column. On either side and connecting with these bundles the bundle representing half the midrib of the adjacent fertile carpel which has also turned inwards. In the centre the ten-fluted style column with ten vascular bundles in line with the grooves, each bundle representing half the vascular system of a sterile member conjoined with half the vascular system of the adjacent fertile carpel. Within the ring of bundles five tracts of conducting tissue, on the radii of the loculi and in line with alternate arcs in the outline of the column. Fig. 32. The staminal tube with its five pairs of vascular cords enclosing the ten-fluted style column in which the separate tracts of conducting tissue seen in Fig. 31 have now coalesced into a solid core. Vascular bundles as in Fig. 31. Fig. 33. Upper region of the style column where all but two of the vascular bundles seen in Figs. 31 and 32 have become resolved into their two components. The core of conducting tissue seen in Fig. 32 has now become divided into ten separate tracts. Fig. 34. Summit of the style column showing stages in the delimitation and separation of the ten stigmatic filaments corresponding to the five sterile and five fertile carpels. Each filament with the twin bundles of the corresponding midrib and a tract of conducting tissue. Fig. 35. Malopeae. *Malope trifida* Cav. Flower base after exertion of the epicalyx-calyx ring showing disjunction of the corolla-androecium tube from the gynaeceum on the radii of the petals and the consequent development on these radii of the first ovaries in the succession. Around the central parenchyma the ring of bundles serving the whole series of ovaries. All from transverse sections taken when in series from below upwards. All magnified equally except Figs. 29-32 and 35, which are less highly magnified than the rest.

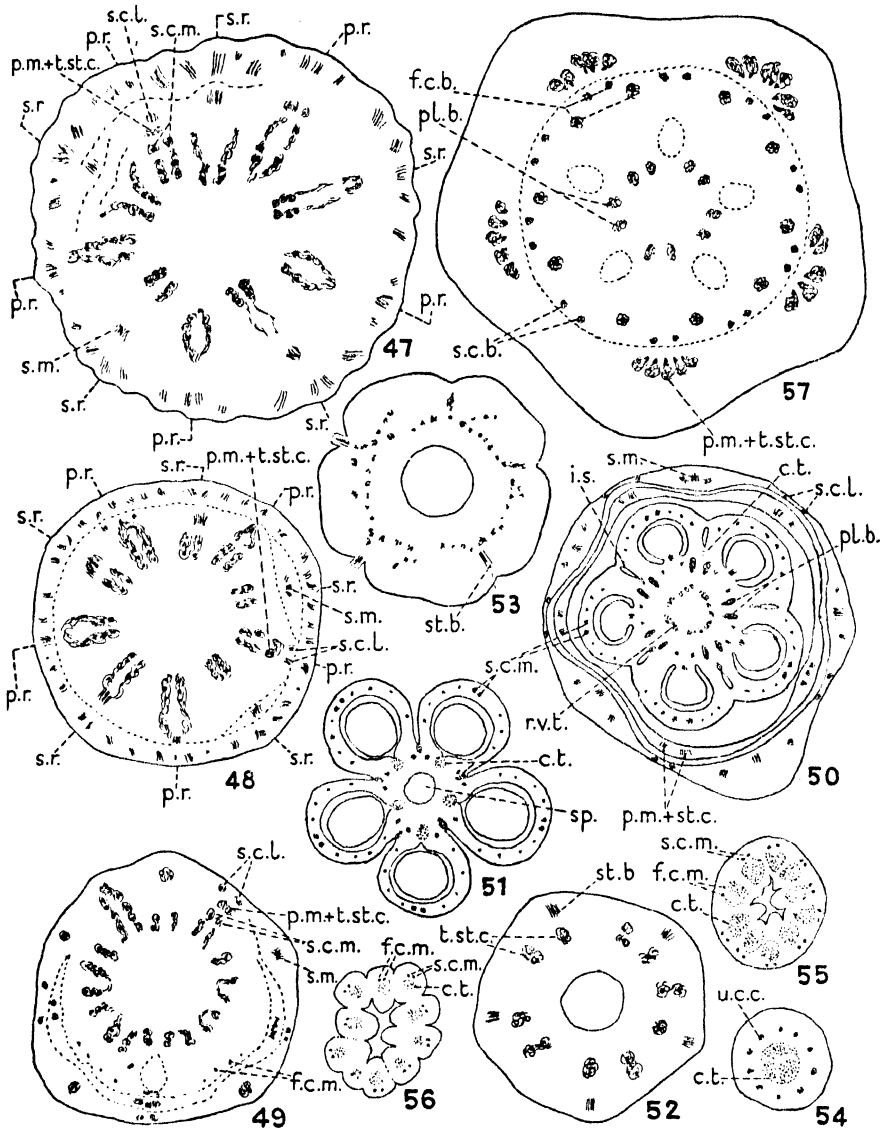


actuality to be accepted, for in giving the further detail that the five without loculi remain sterile he interpolates 'if I may express myself thus' (*si je puis m'exprimer ainsi*) and adds that they prolong in some manner (*en quelque sorte*) the septa. Thus, although reaching the very verge of the conception of carpel polymorphism, Payer evidently hesitates to commit himself to so unorthodox an interpretation; and although he gives a figure of *Urena*, and therefore supposedly also examined this genus, he gives no indication that in its gynaeceum the same relations are to be found as in *Pavonia*, although, in fact, all the *Ureneae* have this feature in common.

*Malvaviscus mollis* DC. Epicalyx (in the specimens examined) 6-9. Here, as in *Pavonia*, the want of conformity between the epicalyx and calyx ground-plans disturbs the symmetry of the vascular ground-plan at the flower base. In its main features the vascular scheme of the succeeding whorls is similar to that described above for *Pavonia*. The midrib bundles for both carpel whorls take the form of twin strands, those for the sterile members being differentiated from the same outgoing cords as the conjoined petal-stamen bundles, hence the loculi here too originate in line with the petals. But as development proceeds torsion of the corolla-androecium tube presently causes the loculi no longer to stand strictly in line with the petals. Herein we have the explanation of the contradictory statements of different writers respecting the radial position of the loculi in this and other genera in this section. Except for its slightly 'gynobasic' origin the style column shows the same features as in *Pavonia*, at first a ten-fluted outline

FIGS. 36-46. *Ureneae*. Figs. 36-45. *Pavonia multiflora* A. St. Hil. Fig. 36. Flower base above the level of exertion of the conjoined epicalyx and calyx (left in position) but before the corolla-androecium tube, though delimited, has become disjoined from the gynaeceum. In line with the petal-stamen cords the sterile carpel midribs, each of two bundles  $\pm$  lateral veins; on the alternate radii the similar midribs and the twin placental bundles of the fertile carpels. Fig. 38. Three of the five loculi have made their appearance and stand in line with the petals. Fig. 37. The gynaeceum showing all five loculi now fully developed. [For simplicity the ovules have been omitted here and in Fig. 46.] Connecting with the internal angle of each loculus a tract of conducting tissue. In the centre a space formed as the parenchyma begins to come to an end. Carpel bundles as in Fig. 36. Fig. 38. The corolla-androecium tube and ovary just below the level at which the loculi close. The twin bundles of the fertile carpel midribs are seen cut obliquely as they turn inwards between the loculi in their course to enter the style column. In line with the loculi, but at this level not in connexion with them, five tracts of conducting tissue. Fig. 39. The corolla-androecium tube and five-sided style column. In the latter five pairs of vascular bundles alternating with the five tracts of conducting tissue. Each bundle represents half the vascular system of a sterile carpel conjoined with half that of the adjacent fertile carpel. Fig. 40. The staminal tube after disjunction from the petals together with the style column now circular in outline. Vascular bundles and conducting tissue tracts as in Fig. 39. Fig. 41. The same at the level at which individual filaments are becoming free. Fig. 42. The style column now becomes ten-fluted with the ten vascular bundles seen in Figs. 39 and 40 in line with the grooves. In two rings round the central parenchyma ten tracts of conducting tissue in line with the ten arcs in the outline, the five outer corresponding with the five sterile, the five inner with the five fertile carpels. Fig. 43. The same after each of the ten vascular bundles seen in Fig. 42 has become resolved into its two components. Fig. 44. The same after these components have diverged, so that a pair representing the twin bundles of one midrib now stand in line with each arc in the outline and each tract of conducting tissue. Fig. 45. The same at the level at which the column begins to give rise to the ten stigmatic filaments. Fig. 46. *P. spinifex* Cav. The ovary at a level between those represented in Figs. 37 and 38 showing already ten tracts of conducting tissue, five outer in line with the sterile, five inner in line with the fertile carpels. All from transverse sections taken when in series, from below upwards. Figs. 42-45 more highly magnified than the others in this series.





FIGS. 47-57. *Urena*. Figs. 47-56. *Urena lobata* L. Fig. 47. Flower base at the level at which the boundaries of epicalyx, calyx, and corolla-androecium tube are becoming defined, showing stages in the organization from each vascular complex on the sepal radii of commissural laterals for the bracts, sepal midrib, and twin strands representing the midrib bundle of a fertile carpel; from each of those on the petal radii, sepal commissural laterals, petal-stamen trunk cord, and twin strands representing a sterile carpel midrib. Fig. 48. The same from about the same level from another flower with epicalyx wholly, and calyx partly, defined. Fig. 49. The same after exertion of the epicalyx and partial delimitation of the calyx and corolla-androecium tube. One locus is now defined standing in line with the strands forming a petal-stamen cord. Fig. 50. The same showing complete disjunction of calyx, corolla-androecium tube, and gynaecium. The fertile carpels are beginning to split in the median plane from without inwards and also interstitially. At the inner end of the interstitial split a small tract of conducting tissue. In the central parenchyma undifferentiated vascular elements not utilized in carpel formation. Fig. 51. The gynaecium. In line with each

with ten vascular bundles in line with the grooves and five tracts of conducting tissue in line with each alternate arc ; later a more or less solid core of conducting tissue, and finally ten pairs of bundles and ten tracts of conducting tissue.

*Urena lobata* L. Epicalyx 5-fid. After the formation of the vascular bundles for the epicalyx, which here is exerted separately, the vascular ground-plan becomes diagrammatic in its regularity. A transverse section shows a ring of ten U-shaped cords with the open ends directed towards the axis centre. The elements in the curved region of the U become delimited in each cord from those forming the 'limbs'. This delimited portion in the case of the five cords on the sepal radii turns outwards to become a sepal midrib. The corresponding delimited portion of each cord on the alternate radii breaks up, giving rise to commissural laterals for the two neighbouring sepals, a petal-stamen trunk cord, and the twin strands which become the midrib of a sterile carpel. The twin strands which form the fertile carpel midribs arise from the corresponding original U-shaped cords on the sepal radii but, as in *Pavonia* and *Malvaviscus*, they are delimited and turn out independently and hence take up their position later than those of the antepetalous carpels. Remaining in the centre is the residue of the ten pairs of 'limbs' of the original ten U-shaped cords. The elements in the two 'limbs' of each antepetalous pair curve away from each other and so come to meet, and fuse with, the near-by elements of the antepetalous 'limb' on each side. Each pair of these reconstituted bundles on each sepal radius becomes organized into the twin placental strands of the corresponding fertile carpel. In the meantime some elements of these fused pairs turn inwards into the central parenchyma, remain undifferentiated, and there come to an end. The occurrence in *Urena* of twin bundles (the 'limbs' of the U) on the radii of the sterile carpels, similar in form and position to those on the fertile carpel radii which are eventually organized into the placental bundles, suggests that in some ancestral form of this genus

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loculus as well as at the inner end of each interstitial split, now become continuous with the exterior, a tract of conducting tissue. In the centre an irregular space formed as the central parenchyma begins to come to an end. Fig. 52. Basal region of the staminal tube. Fig. 53. Apical region of the same showing the final break-up of the five pairs of staminal cords serving each antepetalous sector. Fig. 54. Base of the cylindrical style column showing a ring of ten unresolved carpel cords, each consisting of half the vascular system of a sterile carpel conjoined with half the system of the neighbouring fertile carpel. Fig. 55. Upper region of the same after the ten vascular cords seen in Fig. 54 have become resolved into their components, which have diverged so that now the twin bundles on each radius represent the midrib of the carpel on that radius. In the centre the styler canal which appears after the solid core of conducting tissue seen in Fig. 54 separates into ten tracts corresponding with the ten carpels. Fig. 56. The same at the level at which the column begins to give rise to the ten stigmatic arms. Vascular bundles and conducting tissue as in Fig. 55. Fig. 57. *Goethea strictiflora* Hook. Flower base after exertion of epicalyx and calyx. At the periphery the corolla-androecium tube with five petal-stamen cords, defined but not yet disjoined from the gynaeceum. In line with these cords the paired bundles of each fertile carpel midrib and nearer the centre the corresponding twin placental strands. On the alternate radii the twin bundles of the sterile carpel midribs and the loculi. All from transverse sections taken when in series from below upwards. Figs. 54-6 more highly magnified than the others in this series.

both sets of carpels may have been fertile, and that although the antepetalous carpels in the plants of to-day are no longer fertile their placental bundles still persist.

An indentation in the outline and a radial interstitial split in the mid-line of the fertile carpels make their appearance soon after the ovary becomes disjoined from the corolla-androecium tube, indentation and slit gradually extending until they become continuous (Figs. 49–51) as in various other pseudo-apocarpous types, e.g. certain genera in Rutaceae and Cneoraceae (see 11, pp. 648, 649, and Figs. 4–6, 13, 16, 17, 147, 148). The appearance of ten tracts of conducting tissue is to be observed here in the lower part of the ovary (see Figs. 50 and 51) as in *Pavonia spinifex* (compare Fig. 46). As the ovary passes into the style column the separate tracts become continuous, forming a solid core, to separate again towards the apex as in *Malvaviscus* and *Pavonia*. The transition from an arrangement of ten unresolved vascular cords in the basal region of the column to ten pairs of bundles near the summit also takes place in the same way as in these two latter genera (compare Figs. 54–6 with Figs. 31–4 and 39–45).

*Goethea strictiflora* Hook. As noted above, all accounts describe the loculi as being antesepalous, although the floral formula here is the same as in the three preceding genera in which they have been clearly shown above to be antepetalous. The clue to this difference, as might be expected, is to be found in the different vascular scheme.

Here the four bracts of the epicalyx are exerted separately and below the calyx level, thus permitting reconstruction of the central cylinder before the emergence of the sepal bundles. After these latter bundles have turned outwards the whole of the residual vascular tissue, unlike that of the three preceding genera, is concentrated in five ring-shaped (as seen in transverse section) cords standing on the petal radii. The elements in the outer sector of each ring turn outwards to furnish a petal-stamen trunk cord. The remainder, forming the bulk of these rings, now give rise to a number of strands arranged irregularly in a circle and standing both on the sepal and the petal radii. These strands become organized into the pairs of bundles constituting the alternate midribs of the sterile and the fertile carpels. Since both whorls of paired bundles here turn out independently, it is natural that the first to be formed after the emergence of the petal-stamen cords should be those on the sepal radii, and hence that the loculi should be antesepalous. Here the central parenchyma persists almost to the top of the ovary. As it comes to an end the ovary becomes unilocular, and the conducting tissue which at this level lines the channel leading from the loculi into this central cavity now extends over the inner free face of the fertile carpels so as to form a continuous lining. At the same time these members begin to split in the middle line from their now free inner face outwards. After the ovary has passed into the style column successive transverse sections show pre-

cisely the same series of stages as those already described in the preceding types. At first a ten-fluted outline with ten vascular bundles in line with the grooves and a central core of conducting tissue. At a higher level these bundles divide into two; the two halves diverge, so that now a pair of bundles stands in line with each arc of the outline. At the same time the conducting tissue comes to form ten separate tracts, so that as the whole column gives rise to the ten stigmatic filaments each filament has the twin bundles of the midrib and the tract of conducting tissue proper to the individual carpel of which it is the prolongation.

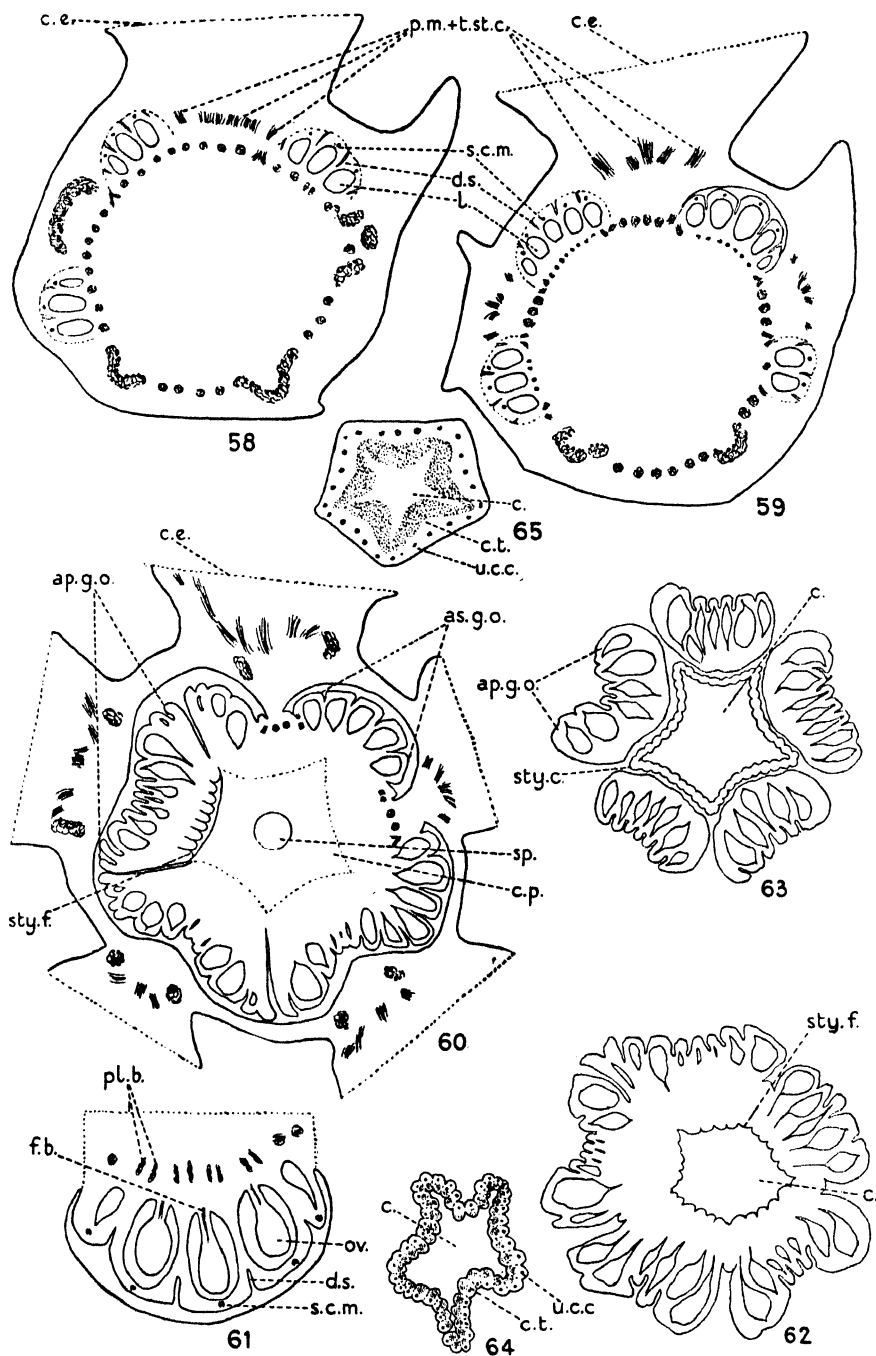
*Malopeae* (Figs. 35, 58-65).

Ovaries  $\infty$ , eventually superposed in numerous vertical rows. Style filaments 'gynobasic', equal in number to the ovaries and loculi.

In this section the disposition of the ovaries has been the subject of much discussion in the past, but no satisfactory explanation of their arrangement has hitherto been forthcoming and the problem of their superposition has remained. Apart from this difficulty, there is the further anomaly that the radial position of the five groups or 'fields' into which the whole number naturally fall appears not to be uniform. The descriptions of different writers on this point are, however, as contradictory as those concerning the position of the loculi in the Ureneae (see above, p. 247). It still remains, therefore, to ascertain whether this difference of opinion arises from error in observation or from a real diversity of ground-plan.

In the following account it is made clear that the above diversity of opinion regarding certain features of the gynaeceum is to be attributed to the different stage of development reached at the time that the observations were made. Further, that while the same general principle governs the arrangement of the ovaries in all the genera, the fact that the process of disjunction of the corolla-androecium tube from the gynaeceum may begin on different radii accounts for the reversed position of the groups of ovaries in some genera, as, e.g., in *Kitaibelia* and *Malope*, which may be taken as illustrative types.

*Kitaibelia vitifolia* Willd. After the organization of the epicalyx and calyx bundles the residual vascular cylinder is seen in transverse section to consist of five arcs of separate bundles in front of the sepals and on the alternate radii of five more compact vascular units, which cause a bulge in the outline of the circle on these (petal) radii. The bulk of the elements in the middle sector of these latter vascular units and a separate smaller strand on each flank turn outwards and give rise respectively to a petal midrib and the twin cords supplying the twin series of stamens standing in front of each petal. The residual strands of these five vascular units now become organized into arcs of separate bundles precisely comparable with those already



FIGS. 58-65. Malopeae (see also Fig. 35). Figs. 58-64. *Kitaibelia vitifolia* Willd. Figs. 58, 59. Flower base after exsertion of epicalyx, calyx, and one petal showing progressive stages in (a) the disjunction of the gynaecium from the corolla-androecium tube, which begins at five points

existing on the sepal radii, so that the whole number of antesepalous and antepetalous bundles are now disposed in a continuous and regular circle. Meanwhile, the development of the ovaries has already commenced. While each petal-stamen complement of strands is still in process of emerging from the central cylinder the midrib bundles of some of the sterile carpels have already turned outwards. One midrib bundle is eventually developed from each of the 60-75 or more separate bundles composing the central ring. The residual portions of each of these separate bundles diverge until portions of neighbouring and originally separate bundles come into contact and coalesce on the intermediate radii. These latter reconstituted bundles, on grounds of analogy and also in view of the whole morphological evidence, are regarded as the fertile bundles of an inner whorl of carpels. These fertile carpels lack the twin strands representing the midrib present in the corresponding carpels of most members of the other sections. The gynaecium thus consists here, as in the rest of the family, of two alternating carpel whorls, the outer sterile, the inner fertile, but the formation of the individual ovaries shows several unusual features. In the first place, owing to the very large number formed, they come into being *in a continual succession instead of simultaneously*. This succession starts with those standing in the mid-line of each sepal. Those at these five points are followed in each case by the next on each side, and so on in regular order, the last to develop being therefore those in the mid-line of each petal. This sequence is in accord with the progressive disjunction of the corolla-androecium tube and gynaecium, which similarly begins in the mid-line of each sepal. The reason for this is

in line with the five sepals, (b) the development of the individual ovaries, (c) the delimitation of the petal, stamen, and carpel vascular bundles. In the outer wall of each ovary the midrib bundle of the corresponding sterile member. Abutting on the central parenchyma on each alternate radius the placental system of a fertile carpel in the form of a single bundle or of twin strands according to the stage in development. In the mid-line of each fertile carpel a radial split extending from without inwards. Fig. 60. The same at the level at which the corolla-androecium tube has become completely disjoined from the gynaecium except on the radius of two petals (above, in the middle line and to the right) showing the division in half of three of the five primary groups of ovaries standing in line with the sepals and with the angles of the central pentagon. On the inner face of the newly constituted antepetalous group of ovaries on the left, now disjoined from the central pentagon or parenchyma, the 'gynobasic' style filaments of the older ovaries. At the periphery the corolla-androecium tube in which the vascular systems of petals and stamens are becoming distinct. [For the sake of simplicity the ovules and all vascular bundles in the ovaries have been omitted in this figure and in Figs. 62 and 63.] Fig. 61. A section of the axis with the attached ovaries at the level of attachment of the ovules, showing the two placental strands of the fertile carpels, one of which furnishes the funicle strand to the solitary ovule. Fig. 62. The gynaecium, after the central parenchyma has come to an end, leaving a space lined with the bases of the 'gynobasic' style filaments. Fig. 63. The same after the division in half of the five original antesepalous groups of ovaries seen in process of taking place in Fig. 60 has become complete, leading to a re-arrangement in five new groups standing against the flat sides of the central pentagon, and so in line with the petals. In the centre the five-angled base of the style column. Fig. 64. The style column shortly before the separation of the component filaments already partly delimited. In each filament a tract of conducting tissue and a single bundle representing the vascular system of a sterile carpel conjoined with half the system of the neighbouring fertile carpel on each side. Fig. 65. *Malope trifida* Cav. The style column showing a belt of conducting tissue not yet separated into as many tracts as there are component filaments, the number of which is indicated by the number of vascular bundles as in Fig. 64. All from transverse sections taken when in series from below upwards. All magnified equally except Figs. 61, 64, and 65, which are more highly magnified than the rest.

soon apparent. At this level, as described above, the petal-stamen vascular complement is still in process of becoming detached from the central cylinder, whereas on the sepal radii organization of the bundles from which the sterile carpel midribs arise is already complete. Hence it is on these radii that the first ovaries begin to take shape, for the outer boundary of the first few in each group becomes defined prior to disjunction of petal-stamen tube and gynaeceal tissue. In consequence they have the appearance of being, as it were, 'carved out' of the surrounding tissue. At this level their lateral boundaries are still only partially defined, for here, as in a number of other families (e.g. Rutaceae, Cneoraceae, Crassulaceae) the apocarpous condition of the mature gynaeceum is of secondary origin, being brought about by the radial splitting from without inwards of the fertile carpels (rendered all the easier here, presumably, by the absence of a midrib in these members). Thus the individual ovaries (the cocci of the fruit) here, as in the Ureneae, are composed of  $\frac{1}{2}1\frac{1}{2}$  carpels.

As the solitary ovules develop, the partially separate ovaries widen and extend further outwards, but they are so closely packed that as each one follows suit it can only enlarge sufficiently by turning obliquely sideways away from its older neighbour on the one side towards, and partly over, its younger neighbour on the other side. That is to say, the successively formed ovaries to one side of the mid-line of each original antesepalous group will successively become inclined from left to right, those on the other side from right to left. Hence each of the five original groups comes to appear divided down the middle as each younger ovary comes to stand at a higher level<sup>1</sup> and at the same time becomes inclined laterally. Consequently, when all the ovaries have developed every two half-groups derived from two neighbouring antesepalous groups have become piled up in contiguous, almost vertical rows, and now appear as one antepetalous group.

The change in the grouping of the ovaries brought about in the manner described explains the contradictory accounts of different observers regarding the position of the groups in relation to the sepal and petal radii and to the angles and sides of the central pentagon of parenchyma. As the succession of ovaries proceeds they become free above from the central parenchyma. This tissue, pentagonal in outline with the angles in line with the sepals in *Kitaibelia*, as it comes to an end, gives place to a five-sided cavity, the boundaries of which are formed by the bases of the numerous 'gynobasic' style filaments. Each of the filaments receives a single vascular bundle. This bundle is formed by the prolongation of the placental system of the corresponding ovary with which the sterile midrib eventually (? always) becomes continuous after it has curved over the top of the loculus from the dorsal to the ventral face. Each filament may be regarded, therefore, as consisting of  $\frac{1}{2}1\frac{1}{2}$  carpels as in the preceding types. Later, in its course up

<sup>1</sup> Through the development of a longer, unexpanded basal region.

the filament, this bundle usually divides into two, sometimes into three, strands. It appears probable that this break-up indicates the separation of the components which went to its make-up.

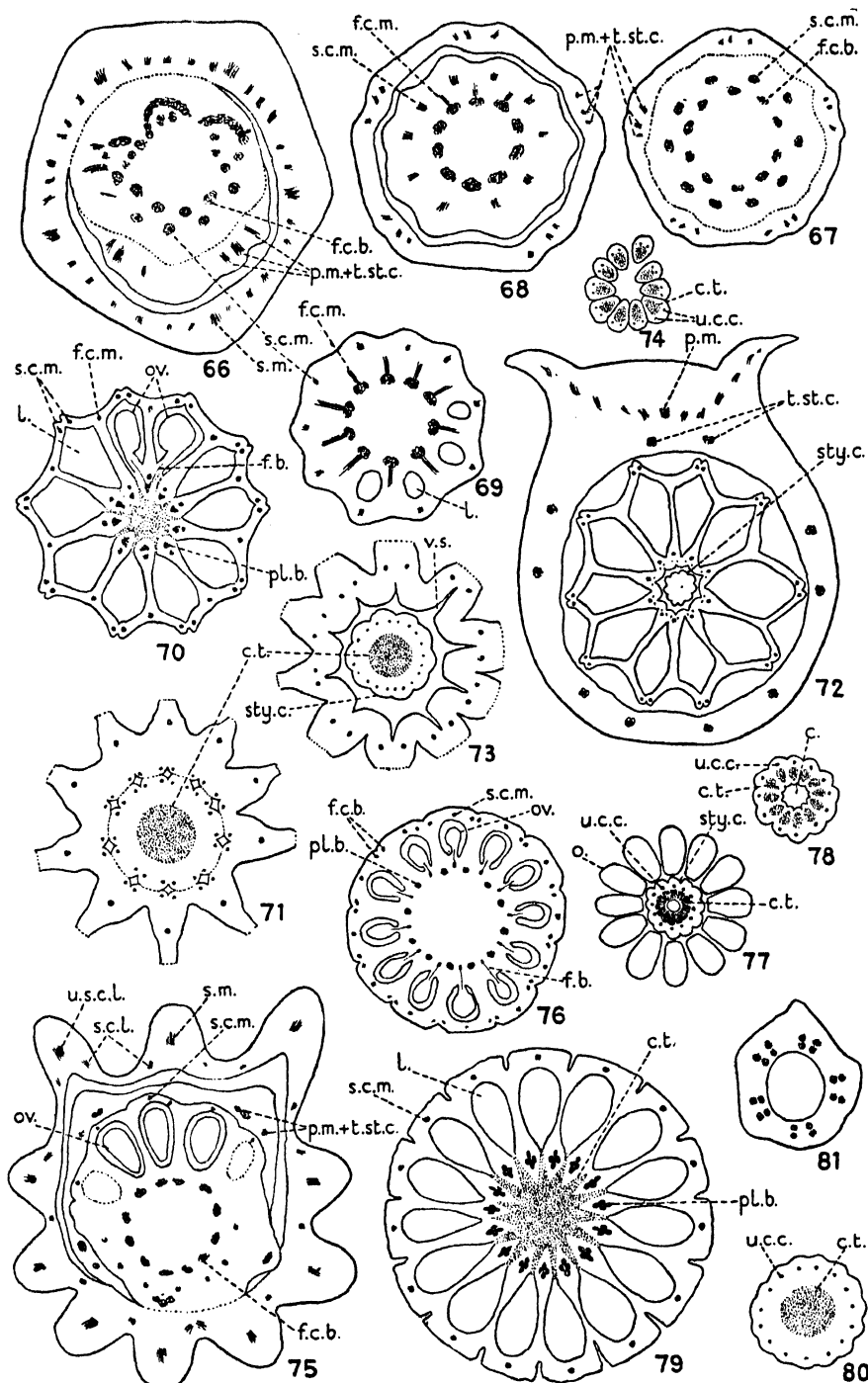
*Malope trifida* Cav. Since the development of the floral members in *M. trifida* follows in general the same course as in *Kitaibelia* it will be unnecessary to describe this type in detail, but the fact that the original groups of ovaries, unlike those in *Kitaibelia*, arise in line with the petals requires explanation. Transverse sections of the flower base reveal the cause of this difference. In *Kitaibelia*, as described above, the base of the corolla-androecium tube first becomes disjoined from the gynaecium in line with the sepals, and only later becomes free on the alternate radii on which the the five petal-stamen groups of vascular strands are still in process of emerging from the reformed central cylinder. In *Malope*, on the other hand, the corolla-androecium vascular bundles do not emerge directly from the central cylinder but are detached from a ring of anastomosing calyx bundles, near the periphery. Consequently, after the departure of the one set of calyx-corolla-androecium bundles the central cylinder is made up entirely of equivalent bundles which, being present in both the sepal and the petal sectors, form a continuous ring. Furthermore, the base of the *Malope* corolla-androecium tube differs in shape from that of *Kitaibelia*. In the last-named genus the non-vascular antesepalous sectors of the tube are much less thick than the alternate sectors in which are the petal midrib and the stamen bundles. In *Malope* the reverse is the case. The antesepalous sectors are thicker than the alternate sectors, and in transverse section are seen to be still connected with the gynaecial tissue by a broad band of parenchyma after disjunction has taken place in the petal sectors. As a result the ovaries on the petal radii develop first and the original groups are therefore here antepetalous.

*Malvace* (Figs. 24, 66-81).

Style column more or less 'gynobasic'. Style branches equal in number to the loculi.

This section includes the bulk of the members of the family of which the following were investigated: *Abutilon Avicennae* Gaertn., *A. vitifolium* Presl., *Anoda hastata* Cav., *Hoheria (Plagianthus) Lyallii* Hook. f., *Lavatera bicolor* Roux, *Malva* spp., *Malvastrum tricuspidatum* A. Gray, *Modiola multifida* Moench. (*Caroliniana* G. Don), *Sida rhombifolia* L., *Sidalcea candida* A. Gray, type and var. *Listeri*, *S. malvaeflora* A. Gray (sterile form), *S. oregana* A. Gray, *S. parviflora* Greene, *Sphaeralcea acerifolia* Nutt., *S. ambigua* A. Gray, *S. Fendleri* A. Gray, *S. munroana* Spach, *S. pedata* Torr. As the relations of the floral whorls in these types are in the main explained by the account which has been given above of the other





FIGS. 66-81. Malveae. Figs. 66-74. *Abutilon Avicennae* Gaertn. Fig. 66. Flower base showing a further stage of development below than above, the calyx being exerted below but merely

sections it will suffice to describe one of the above types in detail, and to refer briefly to individual features in some of the others.

*Abutilon Avicennae* (Figs. 66-74). A transverse section of the flower above the level of emergence of the sepal bundles shows, as in *Goethea* (see above, p. 266), five ring-shaped vascular cords on the petal radii. Elements in the peripheral sector of each ring give rise to a petal-stamen trunk cord. Others forming the sides of the ring become organized into the bundles which supply an outer whorl of sterile carpels. In the typical case of ten carpels each ring will give rise to one such bundle to the right and one to

delimited above, and the corolla-androecium tube being delimited below but not yet defined above where are seen the arcs of vascular elements from which the remaining undeveloped petal-stamen cords are in process of becoming organized and turning outwards. Nearer the centre, below, some of the bundles for the outer sterile and inner fertile carpels already organized. Fig. 67. The same after exertion of the calyx. At the periphery the corolla-androecium tube delimited, but not yet exerted. Near the centre two whorls of bundles serving the outer and inner carpel whorls, respectively. Fig. 68. The same after exertion of the corolla-androecium tube which has been left in position. The sterile carpel midribs (seen cut transversely in Fig. 67) have turned outwards and are seen cut longitudinally. Some of the fertile carpel midribs, which are formed from the central portion only of each bundle of the inner ring, are seen in process of turning outwards in the upper sector. Fig. 69. The gynaeceum after some of the loculi have made their appearance. All the fertile carpel midribs have now turned outwards. Fig. 70. Ovule-bearing region of the same. [For the sake of simplicity only the ovules in one loculus are represented.] The sterile carpel midribs have divided, giving rise to twin bundles. In the centre a core of conducting tissue connecting with the loculi. Fig. 71. The central region of the gynaeceum after the 'gynobasic' style column has become delimited, and in the mid-line of each fertile carpel disjoined, from the inner face of the ovary. The twin placental bundles of each fertile bundle are seen cut twice, once in the fertile carpel before they turn downwards to enter the 'gynobasic' column, and again after they have entered the column and turned upwards. In the centre a core of conducting tissue. Fig. 72. The gynaeceum after the ten-fluted style column (shown in outline only) has become free, surrounded by the exerted androecium tube with which one petal is still conjoined. Fig. 73. The central region of Fig. 72 enlarged to show better the median radial split of the fertile carpels extending from the inner face outwards. In the style column a central core of conducting tissue and a ring of twenty vascular bundles, each bundle consisting of half the vascular system of a sterile carpel fused with half that of the neighbouring fertile carpel. Fig. 74. The style column at the level of separation of the stigmatic filaments. Each filament with a tract of conducting tissue and two bundles, corresponding with the pair seen in Fig. 73, in line with each arc. Figs. 75-8. *Sida rhombifolia* L. Fig. 75. Flower base. Compare with Fig. 66 for stages in the delimitation and exertion of the calyx (here ten-ribbed owing to non-resolution of the primary commissural laterals) and corolla-androecium tube; with Figs. 68-70 for stages in the development of the sterile carpel midribs and loculi. Fig. 76. Ovule-bearing region of the gynaeceum. At the periphery on the one set of radii the undivided sterile carpel midrib bundles, on the alternate set the corresponding fertile carpel bundles now divided in half preparatory to the extension of the median radial split already in progress from without inwards. Nearer the centre on the same radii the placental bundles (single owing to fusion of the twin strands of each carpel) and between them the ovule bundle derived from them. Fig. 77. The crown of the ovary (above the level of the closed loculi) and in the centre the now free 'gynobasic' ten-grooved style column. In line with each arc of the column a single vascular bundle representing the whole system of a sterile carpel and half the system of the adjacent fertile carpel on each side. Within the ring of bundles around a central space (filled at a lower level by the parenchyma of the axis) a ring of conducting tissue partly separated into tracts standing over the loculi and in line with the bundles. Fig. 78. The style column just below the level of separation of the stigmatic filaments which takes place in line with the grooves so that each filament is furnished with one vascular bundle and a tract of conducting tissue. Figs. 79, 80. *Sphaeralcea acerifolia* Nutt. Fig. 79. Middle region of the ovary. [For the sake of simplicity the ovules are omitted.] At the periphery the undivided sterile carpel midrib as in Fig. 76. The fertile carpels in which no corresponding bundles are developed have begun to split in the median plane from without inwards. In the centre the placental bundles and a core of conducting tissue which connects with the loculi. Fig. 80. Base of the style column. Vascular bundles as in Fig. 77. In the centre a core of conducting tissue. Fig. 81. *Sidalcea candida* A. Gray. The androecium tube just below the level of origin of the two rings of phalanges. The twin cords situated in each antepetalous sector have divided tangentially. All from transverse sections taken when in series from below upwards. All those from each species magnified equally, except Figs. 71 and 73, which are more highly magnified than the others in this series.

the left. Of the elements not utilized in these developments some converge towards the centre of the ring and give rise to the bundle supplying one fertile carpel. The rest diverge from the ring to right and to left until they meet and coalesce with the corresponding elements of the neighbouring ring on that side. The bundles resulting from this fusion also become organized into a fertile carpel bundle. In this way the transition in the vascular system is effected, corresponding with the change from a pentamerous ground-plan (calyx and corolla + androecium) to a decamerous ground-plan (gynaecium). For the processes described above result in the formation of an outer whorl of (in the typical case) ten bundles on the perianth radii and an inner whorl of ten on the intermediate radii. The bundles in the outer whorl turn outwards in their entirety and become the midrib bundles of the outer, sterile carpels. Only the central portion of each bundle of the inner whorl turns outwards to furnish a similar bundle for the corresponding fertile carpel, the lateral portions remaining behind to form the twin placental strands. In passing it may be noted that in the development of well-marked midrib bundles in both sets of carpels in *Abutilon* we see another example of the relation to which I have previously drawn attention, viz. that among related forms the less the mucilage (or oil) content the greater the vascular development (see 11, p. 673), for in *Abutilon* the mucilage sacs so abundant in most malvaceous types are lacking. Later, the fertile carpel midribs divide in half preparatory to the median radial split which extends from without inwards. The sterile carpel midribs also divide in half as the ovary wall becomes indented in the mid-line of the loculi. As the loculi become closed these latter pairs of bundles also turn inwards. On their way to enter the 'gynobasic' style column each bundle representing half a sterile carpel midrib, the adjacent corresponding bundle of the neighbouring fertile carpel, and the corresponding placental strand become united into a single bundle. As a result twenty bundles enter the typically decamerous column, and when the style filaments separate each filament receives two bundles, each bundle representing half the vascular system of a sterile carpel conjoined with half the system of the adjacent fertile carpel. Hence in *Abutilon*, as in the *Hibisceae* and *Malopeae*, each filament represents  $\frac{1}{2}1\frac{1}{2}$  carpels, but in *Abutilon* the vascular system is divided up thus:  $\begin{matrix} f.s. & s.f. \\ \frac{1}{2}\frac{1}{2} & (\frac{1}{2}\frac{1}{2}) \end{matrix}$  ( $f.$  = fertile,  $s.$  = sterile).

*Sida rhombifolia*. In this species, as in *Abutilon*, the midrib bundle of the sterile carpels is well developed, but here the ovary wall retains an unindented outline on these radii, and in accord with this difference in contour the midrib bundle remains entire. As the loculi become closed it turns inwards as a single strand picking up in its course the strands representing half the vascular system of the adjacent fertile carpel on each side. Consequently the ten-fluted style column shows only one bundle in front of each arc in the outline instead of the two present in *Abutilon*. Nevertheless, the

vascular complement of style column and stigmatic filament is equivalent in the two genera, corresponding in the *Sida* filament, though represented by only a single strand, to  $\frac{1}{2} + \frac{1}{2}$  capels.

*Sphaeralcea acerifolia*. The calyx of this species shows the unusual feature of ten strong ribs, all having one well-developed vascular bundle, the five bundles on the petal radii representing unresolved pairs of sepal commissural laterals. Here, as in *Sida*, the sterile carpel midrib is well developed and continues undivided up the ovary wall and into the style column, but the corresponding bundle of the fertile carpels, present in *Sida*, is lacking, in accord with the very early stage (as soon as the ovary surface becomes free) at which the radial median split in these members begins from without inwards. As in the last-named type the style column shows a single bundle in front of each arc in the outline. Each of these bundles represents the vascular system of  $\frac{1}{2} + \frac{1}{2}$  carpels. They are continued unresolved into the style branches which, like those of *Sida* but unlike those of *Abutilon*, are furnished with only one bundle.

In the species investigated of *Anoda*, *Hoheria*, *Malva*, *Malvastrum*, *Modiola*, and *Sidalcea*, as in both the two preceding genera, the fertile carpel midrib is well developed and continues undivided in its upward course in the ovary wall. But, as in *Sphaeralcea*, no corresponding bundle is developed in the fertile carpels, the vascular system of these members being reduced to the placental bundles. In all six genera the sterile carpel midribs turn inwards over the closed loculi and, picking up the placental bundle on either side (i.e. half the vascular system of each neighbouring fertile carpel), enter the style column. Consequently the column shows as many vascular bundles as there are loculi and carpels in each whorl, each standing, when the column is fluted, in line with an arc in the outline. In *Lavatera bicolor*, on the other hand, the sterile carpel midrib soon divides in two as in *Abutilon*.

A characteristic feature of both *Anoda* and *Modiola* is the large amount of conducting tissue present. In *Anoda*, owing to the high level to which the central parenchyma is prolonged, this tissue only makes its appearance near the summit of the ovary, just below the level at which the loculi become closed. In transverse section it appears as a large solid core, and extends as such into the base of the style column. In *Modiola*, on the other hand, the central parenchyma comes to an end at the base of the ovary, the corresponding area becoming occupied by conducting tissue. As a result of these relations the characteristic median radial split on the inner face of the fertile carpels, which in most malvaceous genera is seen as a cleft extending into the deeper-lying tissue, appears in cross-section here as a ray in the conducting tissue, the several rays radiating from the centre like the spokes of a wheel. Each ray consists of two well-defined layers of this tissue lining a channel. Presently the conducting tissue ceases to

fill the original area and gives place to a central space with which these channels then connect. At a higher level a fresh set of rays similarly formed on the alternate radii connect the loculi with this space which continues upwards as the stylar canal. Hence the base of the style column, unlike that of *Anoda*, is hollow. In the existence of these two sets of channels we have yet further proof of the presence of two carpel whorls.

It remains to summarize the conclusions to be drawn from the preceding account.

#### SUMMARY AND CONCLUSIONS.

##### (i) *General.*

1. The view held hitherto that the sterile teeth surmounting the staminal tube in certain genera of the Hibisceae and Ureneae represent an antesepalous stamen whorl is disproved. It is shown that these teeth are, in fact, the terminations of the antepetalous sectors of the tube, and that the antesepalous whorl in all genera, including *Sidalcea*, is suppressed without trace.

2. The halving of the individual fertile members composing the five antepetalous groups of stamens goes hand in hand with the separation into two portions of the vascular elements appropriated to each group. This separation is caused by the interpolation (between these two portions) of the large group of elements allocated to the petal standing on the same radius. This spatial relation exists from the moment that the pistil-stamen vascular unit becomes delimited from the central cylinder, i.e. before the determinate phase has been reached. Comparison is made with the similar relation and like result observable in the androecium of the *Fumarioideae*.

3. Repeated subdivision later of the vascular unit serving each half group of already halved stamens, since it occurs after the determinate phase has been reached, is accompanied by multiplication of these members without further fractionization.

4. The gynaecium throughout the *Malvaceae* is shown to be composed, as in all other syncarpous and pseudo-apocarpous types, of two carpel whorls, the outer, being here, as generally, sterile, the inner, fertile. Various apparent anomalies met with in the different sections of the family which till now have remained unexplained are elucidated, including (a) the fact that whereas in the sections *Hibisceae*, *Malopeae*, and *Malveae* the number of separate style filaments is the same as the number of carpels in either whorl and as the number of loculi, in the section *Ureneae* the number of the filaments is twice as many; (b) the fact that in the section *Malopeae*, in which the gynaecium comes to consist of a number of distinct ovaries arranged in the first instance in 'fields', these 'fields' are in some genera antesepalous, and in others antepetalous; (c) the superposition of the

numerous ovaries in all members of the Malopeae one upon another in vertical rows.

5. Where the number of style filaments is equal to the number of carpels in one whorl they stand over the sterile carpels, except in *Gossypium*, where these carpels have almost disappeared ; where the number is twice as many they stand over both sterile and fertile carpels.

6. Median radial splitting of the fertile carpels, such as occurs in greater or lesser degree in pseudo-apocarpous types in other families, e.g. Rutaceae, Cneoraceae, Crassulaceae, gives rise to a collection of cocci, each of which is formed of  $\frac{1}{2}1\frac{1}{2}$  carpels. Exceptionally, the fertile carpels remain entire or incompletely split, the ovary then remains syncarpous, giving rise to a capsule.

(ii) *Respecting the Hibisceae.*

7. In types with an isomerous gynaecium, as e.g. *Hibiscus*, the sterile carpels and loculi are antesepalous.

8. Both the sterile and the fertile carpels possess a midrib bundle. The midrib of the sterile carpels continues throughout as a single strand ; that of the fertile carpels remains single as far as the top of the ovary, but divides in two before entering the style column. The fertile carpels remain unsplit, hence the ovary remains syncarpous.

9. The style column is five-fluted. At its base it has a core of conducting tissue, and in each sector between the furrows three vascular strands. The middle strand is the midrib of a sterile carpel ; the outside strands represent half the vascular system of the neighbouring fertile carpel on each side. Consequently each of the five sectors is composed of  $\frac{1}{2}1\frac{1}{2}$  carpels.

10. The number of style filaments is equal to the number of loculi and of carpels in one whorl, i.e. to half the total number of carpels. Each filament is formed from one of the five sectors of the style column corresponding to  $\frac{1}{2}1\frac{1}{2}$  carpels, but only the two outside vascular strands of the three present in each sector are prolonged into the corresponding filaments, the third, middle strand (the sterile carpel midrib), having come to an end below the level of separation of the filaments.

11. In *Julostylis* and *Dicellostylis* which have an oligomerous gynaecium ( $G\ 2 + 2$ ) the midrib bundles for both carpel whorls, as well as those for the corolla-androecium tube, are detached from an enlarged vascular cylinder and turn inwards.

12. The gynaecium of *Gossypium* is more frequently oligomerous than isomerous. When isomerous it differs from that of other Hibisceae in that the loculi are antepetalous. This position results from the fact that the antesepalous carpels, which in the other genera approach the solid form and are sterile, are here semi-solid and fertile. The assumption of the

reproductive function by the outer whorl which characterizes all forms of the gynaecium (oligomerous and isomerous) has led to the almost complete disappearance of the inner, now functionless, antepetalous whorl. The relation of the *Gossypium* gynaecium in the above respects to that of other isomerous Hibisceae is compared with (*a*) that of the *Astrocarpus* gynaecium, a perfect example of one composed entirely of semi-solid fertile carpels, to the *Reseda* gynaecium, which is composed of solid sterile and semi-solid fertile carpels; and (*b*) with that of *Reseda luteola*, in which the anterior and postero-lateral carpels are solid and sterile, the posterior and antero-lateral semi-solid and fertile to that of *R. odorata*, *R. lutea*, and other six-carpelled species in which there is a similar reversal of form and function, the posterior and antero-lateral carpels being those which are solid and sterile, and the anterior and postero-lateral those which are semi-solid and fertile.

13. The absence of a carpel midrib bundle in line with the loculi, taken together with certain structural features not present in other genera, points to the conclusion (*a*) that if the sterile carpels can be held to exist at all they must be supposed to be represented by a rib of non-vascular tissue projecting into the loculus; (*b*) that the presence of a more or less well-defined radial sheet of cells in the mid-line of this rib indicates the potential surfaces subsequent to a junction or preceding a separation; and (*c*) that the gynaecium above the level at which this rib is traceable must be considered to be composed solely of the semi-solid fertile members. Comparison is made with *Tulipa*, where the sterile carpels, though contracted, have not yet disappeared and the line of contact of the overarching semi-solid fertile members is marked in part by actual surfaces. The conclusion that the outer carpel whorl in *Gossypium* has become suppressed, at least in the upper part of the gynaecium, is further borne out by the form of the stigmas, by the origin and arrangement of the vascular bundles in each individual stigma lobe, and by the configuration of the core of conducting tissue at the stigma level where it extends to the *dorsal* surface, thus defining the individual fertile carpels the inner surface of which it completely clothes.

(iii) *Respecting the Ureneae.*

14. The difference in position of the loculi in different genera having the same ground-plan (*Goethea*, *Malvaviscus*, *Pavonia*, *Urena*) is due to the different manner of origin of the carpel vascular system. In *Pavonia* (? all species) and *Urena* the carpel midribs on the petal radii are derived from the same delimited group of vascular elements as the petal-stamen cords. Hence these midribs are differentiated, and take up their position, before the carpel midribs on the sepal radii, consequently the loculi are antepetalous. In *Malvaviscus* the sterile-carpel midribs similarly arise on

the petal radii, but owing to torsion of the corolla-androecium tube as development proceeds the loculi appear at a later stage not to be situated strictly in line with the petals. In *Goethea*, on the other hand, the loculi are antesepalous since the sterile carpel midribs are delimited and organized independently of the corolla-androecium system, and therefore in normal alternation.

15. The midrib of both sterile and fertile carpels is represented by twin bundles. The fertile carpels become split radially in the median plane, the split proceeding from without inwards between the paired bundles, in *Urena* also interstitially as in some *Rutaceae* and in *Cneoraceae*.

16. The base of the style column is either ten-fluted from the outset (*Malvaviscus*, *Goethea*), or it is at first cylindrical, and shortly becomes ten-fluted (*Pavonia*, *Urena*). It receives five pairs of vascular bundles, which may exhibit for some distance the paired arrangement (*Pavonia*), or may come at once to occupy ten equidistant radii in line with the ten grooves in the outline (*Malvaviscus*, *Goethea*, *Urena*). Each bundle is formed by the fusion of half the vascular system of a sterile carpel conjoined with half that of a fertile carpel. The conducting tissue may consist of five separate tracts which shortly fuse (*Malvaviscus*, *Goethea*, *Pavonia*), or it may appear as a solid core from the outset (*Urena*).

17. The ten vascular bundles in the base of the style column break up at a higher level into their two components. The two halves of one of these bundles stand at first in line with each groove, but later diverge so that the adjacent halves of two neighbouring bundles then form a pair in line with each arc in the outline. In this way the twin strands representing the midrib of one carpel are again brought together so that they lie in the same sector. The core of conducting tissue also breaks up, giving rise to ten separate tracts standing in line with the ten arcs in the outline and arranged in two rings, the outer five being situated on the radii of the sterile carpels, the inner five on those of the fertile carpels.

18. The number of style filaments is twice the number of loculi and equal to the number of carpels in the two whorls, i.e. to the total number of carpels. Each filament is formed of one of the ten sectors of the column, and receives the corresponding tract of conducting tissue and the pair of strands standing in that sector and representing one carpel midrib, hence each filament is formed of a single carpel.

19. Development of separate and similar style filaments by both the sterile and the fertile carpels affords incontestable proof of Carpel Polymorphism. Comparison is made with *Eschscholzia*, in which the filaments of the two types of carpel are also similar in form, and with the section *Paepalanthoideae* of the *Eriocaulaceae*, in which the two sets differ in appearance.



*(iv) Respecting the Malopeae.*

20. The gynaecium consists of a large number of ovaries which become arranged in five 'fields'.

21. The separation of the ovaries comes about through median radial splitting of the fertile carpels which lack a midrib bundle; the splitting proceeds from without inwards and begins at once as the carpels take shape; each ovary is thus formed of  $\frac{1}{2}1\frac{1}{2}$  carpels.

22. The manner of development of the ovaries is unique in that those in each 'field' do not develop simultaneously but in succession. The order of succession is from those on the antesealous to those on the antepetalous radii, or in the reverse direction, according as the shape of the base of the corolla-androecium tube leads to disjunction of the tube from the gynaecium taking place first on the sepal radii (*Kitaibelia*) or on the petal radii (*Malope*).

23. The enlargement successively of so many closely packed ovaries combined with the early splitting from without inwards, in similar succession, of the fertile member of each ovary leads to a certain characteristic change both in orientation and position of the ovaries in each 'field', which explains the contradictory accounts which have been given of their arrangement. This change comes about in the following way. The two oldest ovaries in each of the five original 'fields', i.e. the two nearest the mid-line, turn laterally in such a manner that the fertile carpel common to both becomes more widely split. This causes the outer wall of these two ovaries to overlap that of their younger neighbours, which are in consequence forced to develop at a slightly higher level, while at the same time turning laterally in the same direction as those that preceded them. In this way the five original 'fields' become divided in half vertically, five new 'fields' appear on the alternate radii, and ovaries originally standing side by side at the same level become piled upon each other, one after the other, in almost vertical rows.

24. In each ovary the vascular system of the sterile carpel becomes continuous with that of the two fertile half-carpels below the level of origin of the corresponding sector of the 'gynobasic', hollow style column.

25. The number of style filaments is equal to the number of ovaries, and therefore to half the total number of carpels. Each filament has a tract of conducting tissue, and receives one of the strands representing the vascular system of a sterile carpel conjoined with half that of the fertile carpel on each side. This strand may remain unresolved, or it may eventually split into two or three separate strands. These ultimate strands presumably represent the components which went to the make-up of the original single strand, the number varying, presumably, according as the sterile carpel midrib persists to the end or dies out.

(v) *Respecting the Malveae.*

26. Radial splitting of the fertile carpels takes place usually from without inwards as in the Malopeae, but begins at a later stage owing probably to the presence in these carpels, at the base, if not throughout, of a well-developed midrib bundle which is lacking in the Malopeae. In *Abutilon* the split proceeds also from within outwards.

27. The number of style filaments is equal to the number of the loculi and to half the total number of carpels.

28. The number of vascular bundles, both in the style column and in the whole number of filaments, is in some genera (*Anoda*, *Modiola*, *Sidalcea*) equal in number to the number of carpels in one whorl, and in other genera (*Abutilon*) is twice as many; nevertheless the individual filament in all genera corresponds to  $\frac{1}{2}1\frac{1}{2}$  carpels, as is evident from the facts contained in the two following paragraphs.

29. In types of the *Anoda-Modiola-Sidalcea* class each vascular bundle entering the style column consists of the whole sterile carpel midrib fused with half the vascular system of the neighbouring fertile carpel on each side. All these bundles may continue upwards unresolved, in which case each filament receives a single bundle (*Modiola*, *Sidalcea*); or some bundles may break up and others not (*Anoda*), in which case some of the filaments will receive one bundle and others more than one.

30. In types of the *Abutilon* class the sterile carpel midrib splits in two in its course up the ovary wall. These halves remain distinct from each other, but each fuses with half the vascular system of the neighbouring fertile carpel before entering the style column. Every filament receives a pair of these bundles, every pair representing the two halves of one sterile carpel midrib together with half the vascular system of two fertile carpels.

The accompanying figures were drawn by Miss D. F. M. Pertz, whom I here tender my very grateful thanks. I also desire to thank the following, to whom I am much indebted for material: the Director of the Royal Botanic Garden, Kew, the Directors of the Botanic Gardens at Cambridge and Singapore, the Regius Keeper of the Royal Botanic Garden, Edinburgh, Dr. S. C. Harland, Mr. F. G. Preston, Mr. R. A. Silow, Mr. N. D. Simpson, and Mr. J. S. Yeates.

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# Transpiration and Pressure Deficit.

## IV. The Effects of Small Deficits: Apparatus and Preliminary Experiments.<sup>1</sup>

BY

F. M. HAINES, Ph.D.

With two Figures in the Text.

### INTRODUCTORY.

A GENERAL account of the effects of artificially applied pressure deficits (for definition see 2, pp. 679-80, and 3, p. 213, footnote) on transpiration rate over a wide range of pressures has already been given in earlier papers of this series (3, 4, 5). So far, however, only the effects of deficits of over one atmosphere (15 lb./in.) have been considered. It was found in some of the earliest experiments on the subject in 1927 that very small deficits gave totally unexpected and anomalous results. Before causing the reduction of transpiration which is normally brought about by larger deficits, small deficits appear to cause a preliminary *increase* in transpiration rate followed by a decrease.

### EXPERIMENTAL.

The method employed for the investigation of these effects was that already outlined and described as 'method (a)' (3, pp. 213-14). The results to be described are those to which allusion is made on p. 214, § 4 (3).

A cut leafy branch (detached under water with the usual precautions) was inserted through the rubber stopper in the shorter limb, A, of a glass vessel of the shape shown in Fig. 1. The whole vessel was completely filled with water, the longer limb being closed at the top by a rubber stopper carrying a capillary tap. Inside the vessel was placed a special type of manometer for registering the reduction in pressure in the vessel.

The manometer consisted of a J-shaped glass capillary tube with a small glass bulb on the shorter limb approximately 1 cm. in diameter. The bulb was half filled with air, the lower half of the bulb and the glass bend containing mercury. The long limb was graduated and was open at the top. Reduction of pressure in the vessel causes the mercury to rise over the scale. Before use the instrument was calibrated by placing in a vessel connected with an ordinary mercury manometer and gradually exhausting the vessel with a pump.

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The whole apparatus was suspended in the centre of a wind-tunnel, being allowed to hang from the beam of a balance mounted on the top of the tunnel. The tunnel was of wood with glass windows for observation and did not have to be opened for weighings. The balance in some experi-

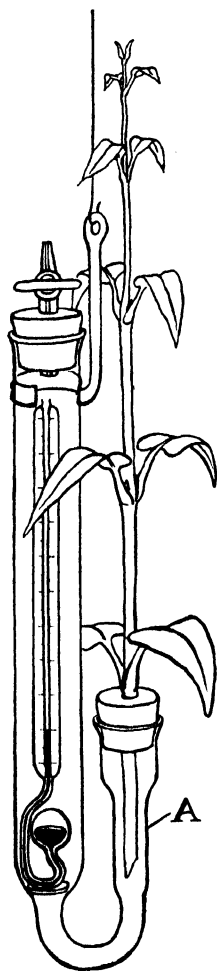


FIG. 1. Apparatus for following changes in transpiration rate with increasing small pressure deficit. For explanation see text.

ments was self-recording. A frame of electrically heated nichrome wire coils was placed in the mouth of the tunnel, and a toluene gauge with a very thin elongated bulb coiled in the plane transverse to the axis of the tunnel was placed about three feet from the heating coils. The air was kept just moving by means of an electric fan at the opposite end of the tunnel, only just sufficient draught being allowed to ensure the satisfactory operation of the thermostatic arrangements. The temperature was constant within  $1^{\circ}\text{C}$ . Humidity was not controlled, but was shown by means of a Cassella hair hygrometer not to vary beyond 1 per cent. during any one experiment, the experiments being of relatively short duration.

In each experiment the branch was cut under water and the last two inches at the base were stripped (under water) down to the wood. The branch was then inserted through the rubber stopper of the limb, A, of the apparatus (Fig. 1) by the usual method of passing it down an oversized cork-borer previously inserted through the stopper from below and subsequently withdrawing the borer. This was done in such a way as to leave only intact stem protruding above the stopper and only stripped stem below, the end of the bark being as nearly as possible half-way up the bore of the stopper. This more or less completely prevented the forcing of air down the intercellular spaces on reduction of the pressure in the vessel. The stopper and branch were then fixed in the short limb of the vessel, while the latter was held under water in a large trough. The manometer was inserted, the vessel filled with water, and the rubber stopper and tap placed in the top. The outside of the vessel was thoroughly dried, and the whole hung

on the balance in the wind-tunnel with the tap open until the weighings showed that the rate of water loss had become constant. This usually required from one to two hours.

When the rate of transpiration had been constant for half an hour, the constant rate was noted, the vessel refilled with water to the bore of the tap,

and the tap turned off. Readings of the transpiration rate as shown by the balance were taken at intervals against the falling pressure in the vessel. The reduced pressure in the tracts at the base of the stem was taken as equal to that in the vessel and therefore to that indicated by the manometer. As very little air was present in the vessel at the beginning of the readings, it was not considered necessary to make any correction for the buoyancy effect of the partial vacuum produced as the water was absorbed. This effect would tend to increase the apparent rate of loss in weight when the tap was turned off, since the water absorbed from the vessel could not then be replaced by air, but it can easily be shown to be altogether incapable of accounting for apparent increases of the order found in the experiments. The mass of a litre of air at 18° C. and 760 mm. pressure is 1.213 gm. Consequently the buoyancy of 5 c.c. of total vacuum could only amount to  $\frac{5 \times 1.213}{1000}$  gm. or 0.006065 gm. If, therefore, water were absorbed at a rate of 5 c.c. per hour, which is the approximate rate noted in the experiments, the error due to this effect would be at the most 0.006065 gm./hr., or 0.01213 per cent. The increases recorded range from 25 to nearly 300 per cent.

The results of some experiments on *Eupatorium adenophorum* are given in Table I.

It will be seen that when a small pressure deficit is first created there appears usually to be an initial increase in the rate of evaporation which later falls off. This is shown by all the experiments (Table I) except Experiments 8 and 10. The experiments were discontinued at the first signs of intercellular leakage of air.

In a few cases where the difficulty of leakage of air into the vessel through the intercellular space system did not develop so soon it was possible to continue the experiment over a longer period of time. A self-recording balance was used to follow up the changes in evaporation rate. Steel balls were automatically delivered into the balance-pan to compensate for the loss of water, which was electrically recorded on a drum. The individual balls weighed 0.1306 gm., twelve divisions per inch on the record corresponding to a rate of water loss of 1.1 gm. per hour. The result of a typical experiment of this kind is shown in Table II. The initial rise in evaporation rate is again evident, being maintained in the case shown for nearly two hours, or until the deficit reached approximately 15 cm. of mercury. (The last readings show the usual recovery effect already described as occurring during higher deficits (4, pp. 551-2).

A further series of experiments was performed on woody branches of Horse Chestnut (*Aesculus*) and Lime (*Tilia*). The cut branch, after standing one hour in water, was simply allowed to take up water from a closed test-tube fitted with a rubber stopper through which the branch was fixed and

TABLE I.

*Transpiration Rates of Eupatorium adenophorum under Small Increasing Pressure Deficits.*

Expt.	Temp. °C.	Pressure deficit (cm. Hg).	Transpira- tion rates as per- centages of initial rate with atm. press. in vessel.	Expt.	Temp. °C.	Pressure deficit (cm. Hg).	Transpira- tion rates as per- centages of initial rate with atm. press. in vessel.
1	17.5	0.0	100	6	20.0	0.0	100
		9.0	145			4.0	233
		18.0	81			7.5	195
		19.0	58.5				
2	18.0	0.0	100	7	21.0	0.0	100
		1.0	100			5.4	174
		7.5	125			9.0	139
		11.5	112				
		14.8	97.5				
3	19.0	0.0	100	8	21.0	0.0	100
		3.6	375			5.5	70.5
		4.5	200			8.5	70.5
4	20.0	0.0	100	9	21.0	0.0	100
		2.0	161			3.3	142
		4.5	167			12.0	177
		4.7	163				
5	20.0	0.0	100	10	21.0	0.0	100
		0.75	125			5.0	76.5
		2.0	107			6.5	53.3
		3.5	104				

TABLE II.

*Experiment II. (Eupatorium adenophorum.)*

Time from beginning of experiment.		Pressure deficit in cm. of mercury.	Transpiration in divisions per inch on drum record.	Transpiration rates as gm. of water lost per hour.	Transpiration rates as percentages of initial rate.
Hr.	min.				%
0	0	0.0	18.3	1.65	100
0	30	0.0	18.3	1.65	100
0	45	0.0	18.3	1.65	100
Tap turned off:					
1	0	3.0	20.0	1.80	109
2	0	12.0	19.0	1.71	104
4	0	22.5	15.0	1.35	81.8
4	25	24.0	13.5	1.215	73.8
4	42	25.0	11.5	1.035	62.8
5	0	26.0	11.0	0.99	60.2
6	0	27.0	11.5	1.035	62.8
7	0	27.5	11.5	1.035	62.8
8	0	28.0	11.5	1.035	62.8

made perfectly air-tight. Absorption then rapidly created a natural deficit. The base of the branch was prepared and inserted through the stopper in the way already described (p. 284 above) and the tube completely filled with water prior to fitting in the stopper and branch. During the insertion of the stopper a piece of thin wire was held down the inside of the tube to prevent any excess pressure being set up in the tube. When the stopper was well home the wire was withdrawn, leaving it perfectly airtight. The whole system was then hung upon the balance and the rate of water-loss plotted against time during the development of a pressure deficit in the tube and tracts. The rate of water-loss can be most accurately and conveniently followed up by noting the times at which the balance-pointer crosses the zero mark after the successive removals of equal small weights (e.g. 0.01 gm. at a time) from the counter-balancing pan.

The system is first balanced, the time noted, and the first 0.01 gm. taken off (special stops being provided under the beam to prevent undue deflexions). The exact time is then noted at which the pointer passes the zero mark of the scale, indicating the loss of 0.01 gm. of water. The beam is then momentarily rested and a further 0.01 gm. removed. The time is again noted at which the loss of the next 0.01 gm. of water is completed in the same way.

In this way the times are recorded at which successive equal increments of water are lost, and the usual error due to the time required to take the weighings is eliminated. The wind tunnel was not used in these experiments, the plant being protected from draughts by large sheets of mill-board. Later series of experiments in a glass transpiration chamber with an externally operated balance have given similar results. The results of two typical experiments are given in Tables III and IV.

The initial increase was again evident in all experiments performed.

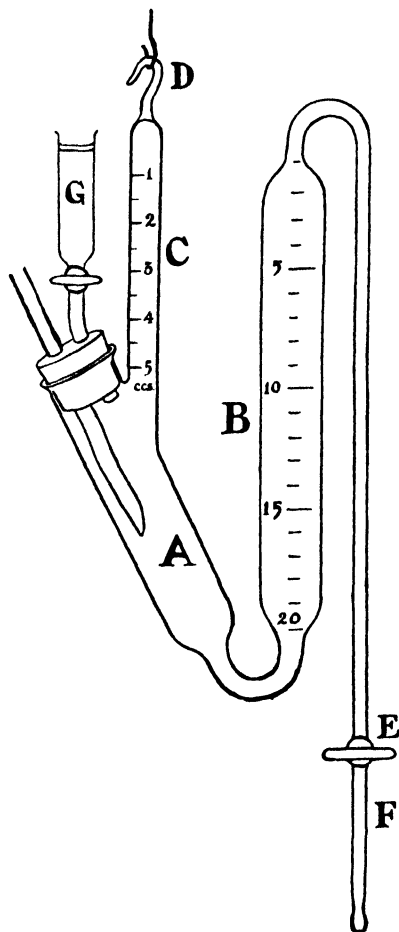


FIG. 2. Apparatus for following transpiration and absorption rates under increasing small pressure deficits. For explanation see text, p. 288.



The last reading in Experiment 12 is erratic, evidently owing to slight leakage of air through the pith at this point causing a slight decrease in the deficit. It has already been shown (4, p. 552) that a small decrease in the deficit if applied (as here) at a time when the transpiration rate is reduced by the deficit normally causes an increase in evaporation rate. The *immediate*

TABLE III.  
*Experiment 12. Acer.*

Time in minutes.	Mass in gm.	Interval between arrows minutes.	Loss during interval in gm.	Rate of loss in gm./hr.
→ 0	24.70			
5	23.85	12	1.9	9.5
→ 12	22.80			
13	22.50			
14	22.30			
15½	22.0			
17	21.7	10	2.3	13.8
18	21.5			
19	21.2			
21	20.7			
→ 22	20.5			
25	20.0	10	2.0	12.0
27	19.5			
→ 32	18.5			
37	17.6	6	1.0	10.0
→ 38	17.5			
→ 58	15.0	20	2.5	7.5
79	14.0	30	1.5	3.0
→ 88	13.5			
→ 97	13.0	9	0.5	3.3

effect of a small decrease in the deficit, however, if occurring during the initial period when the transpiration rate is increased by the deficit, is, as seen in Experiment 13 (Table IV), to cause a slight *decrease* in transpiration rate. The last interval in this experiment (Experiment 13) was sufficiently short for the initial decrease in transpiration rate to be observed, and the decrease in the deficit (unlike that in Experiment 12) was allowed to take place during the initial period of increased transpiration rate.

Further series of experiments were conducted with the type of apparatus shown in Fig. 2. This allows of the simultaneous measurement of transpiration and absorption rates and also of leaf water contents. It is designed to allow the volume of water absorbed to be measured in spite of the accumulation of bubbles which takes place when the water is under reduced pressure. It consist of a reservoir, A, and two graduated limbs, B and C. It is suspended by the glass hook, D, from the beam of the balance, the parts being so shaped and proportioned that the limbs B and C hang vertically during experiments. A cut branch with stripped base is fixed, as described above, (p. 284) through a rubber stopper in the mouth of the reservoir, A, the base

of the stopper when inserted coming level with the base of the vertical limb, C. Any bubbles of air or vapour arising in A as a result of the reduced pressure then rise into C, where their volume can be read off and the necessary

TABLE IV.

*Experiment 13. Acer.*

Time min. sec.		Mass in gm.	Interval between arrows min. sec.		Loss during interval in gm.	Rate of loss in gm./hr.
→ 0	0	64.60				
→ 2	30	64.55	2	30	0.05	1.2
4	30	64.50				
6	30	64.45	6	0	0.15	1.5
→ 8	30	64.40				
10	20	64.35				
12	20	64.30	7	50	0.20	1.53
14	20	64.25				
→ 16	20	64.20				
18	20	64.15				
20	10	64.10	7	45	0.2	1.55
22	10	64.05				
→ 24	5	64.00				
26	10	63.95				
28	5	63.90	8	0	0.2	1.50
30	10	63.85				
→ 32	5	63.80				
34	0	63.75				
36	0	63.70	8	2	0.2	1.50
38	5	63.65				
→ 40	7	63.60				
42	3	63.55				
44	3	63.50	7	58	0.2	1.51
46	5	63.45				
→ 48	5	63.40				
50	5	63.35				
52	5	63.30	7	55	0.2	1.52
54	5	63.25				
→ 56	0	63.20				
58	0	63.15				
60	5	63.10	6	5	0.15	1.48
→ 62	5	63.05				
→ 98	5	62.25	36	0	0.80	1.33
105	5	62.10	34	10	0.70	1.23
→ 132	15	61.55				

Vessel opened and deficit eliminated—

→ 135	20	61.50	3	5	0.05	0.97
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correction applied for the volume of water absorbed. At the beginning of each experiment the vessel is completely filled with water up to the zero mark at the top of the burette, B. This is facilitated by the use of the separating funnel, G, which is later refilled, if necessary, and closed with a thin layer of oil to prevent evaporation. The funnel, G, also permits the apparatus to be reset during experiments. Finally, the whole apparatus is thoroughly dried and hung on the balance by the hook, D.

The rate of evaporation is then followed up against time by weighings taken in the manner already described (p. 287), and the absorption followed up simultaneously by the fall of the meniscus in B. When readings of the transpiration and absorption rates have been obtained under conditions of zero deficit (i.e. with atmospheric pressure in A), the meniscus in B is returned to the zero mark by letting in water from the separating funnel, G; the tap,

TABLE V.

*Experiments on Aesculus hippocastanum, the Periods under Reduced Pressure being approximately 3/4 hr.*

No.	Pressure deficit.	Transpiration rate.	Absorption rate.
1	0	5.0	5.0
	25 cm. Hg	6.1	5.25
	0	5.2	5.5
2	0	1.2	1.2
	50 cm. Hg	1.4	1.1
	0	1.0	1.1
3	0	6.45	6.45
	50 cm. Hg	9.65	8.62
	0	8.7	8.7

E, is turned off and readings of the transpiration and absorption rates are again taken during the development of a pressure deficit in B. The reduced pressure in the vessel may be allowed to arise naturally as a result of absorption when the tap, E, is turned off, or, if desired, may be produced artificially by evacuating the vessel by means of a pump attached to the tube, F. The actual value of the deficit in these experiments was not measured. If bubbles of air or vapour collect in the apparatus at this stage they are caused to collect in C by gentle tapping, and the volume of vapour in C is added on to the reading in B to give the true volume of water absorbed. When these readings have been attained the tap, E, is opened to eliminate the pressure deficit, and further readings of transpiration and absorption rates are taken at atmospheric pressure. Before this is done, the apparatus may be again reset if necessary by returning the meniscus in B to the zero mark by running in water from G.

By periodically detaching leaves for determinations and making due allowances in the weighings the changes in leaf water content may be simultaneously determined. The variations in leaf water content with deficits will, however, be dealt with in separate communications.

The results of three representative experiments of this type are given in Table V. Provided the first period under reduced pressure upon which the rates are based be not too long (e.g. not more than  $\frac{1}{2}$ –1 hour) the initial increases in transpiration rate are always observed. The absorption rates usually, though not invariably, show a corresponding increase. Should the first period under reduced pressure be too long (e.g. more than  $\frac{1}{2}$ –1 hour)

the initial effect is more than masked by the normal subsequent decrease. After a certain length of time in any experiment the average rate appears to have remained unaltered as compared with the rate under atmospheric pressure owing to the exact cancelling out of the initial increase and the decrease which follows

It will also be noted that during the preliminary period of increased transpiration consequent upon the creation of a small deficit, reduction or elimination of the small deficit leads to a temporary decrease in the transpiration rate.

The general effects of small deficits described in the above preliminary experiments have subsequently been confirmed on various types of material. They have also been recently observed quite independently and without any knowledge of the writer's earlier work by E. C. Humphries, B.Sc., by whom a further account of later work will be published shortly.

The wind tunnel used in the earliest experiments was the one originally used by Blackman and Knight (1) in 1917. It was kindly lent by Professor V. H. Blackman, to whom the writer is duly grateful.

#### SUMMARY.

An apparatus is described for the investigation of the special effects of small pressure deficits on the transpiration and absorption rates of cut woody branches.

Preliminary experiments show that the first effect of a small deficit is to cause an increase in transpiration rate which is later followed by a decrease. Reduction or elimination of a small deficit during the initial period of increased transpiration causes at first a slight decrease in transpiration rate.

An account of confirmatory experiments will appear later.

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# On Photoperiodism and Changes in the Enzymatic System.

BY

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GARNER and Allard (5) have shown that by changing the length of the photoperiod, the onset of the reproductive phase in plants can be accelerated or retarded.

There exists already in the literature certain data concerning the connexion between the succession of the reproductive and of the vegetative phases and the state of the enzymatic system of the plant.

As far back as 1913 Klebs (8) believed it possible that a change in the phases resulting from external factors might be connected with enzyme activity. Coville (4) has tried to establish experimentally the connexion between a premature flowering and the enzymatic system. Ljubimenko and Scheglova (16), in their highly interesting researches on photoperiodic adaptation, have advanced the supposition that there exists a connexion between the photoperiodic reaction and the oxidation-reduction process. Knott (9) in his investigation on the localization of the photoperiodic effect points out changes in the activity of catalase in the growing points. The same author (10) has established the fact that the flowering plants of spinach have in their stem apices a less active catalase than have those that are kept sterile by a short day. According to Knott's data the leaves of spinach when in flower have a somewhat more active catalase than the leaves of the plants not in bloom. In his latest work (11), Knott shows that there is already a change in the activity of catalase in the points of growth of spinach after 2.5, 16.5, 40.5 hours of a lengthened period of light exposure. According to Miller (17) the connexion between the flowering and the activity of catalase is marked in the cabbage.

Light is the active factor in photoperiodism and it is already known that radiation influences enzymes (investigations of Green (10) and others), and that the presence of the so-called photodynamic substances plays an important role in this process. Chlorophyll, as well as other photodynamic substances, increases the action of light upon enzymes (13). Brown and

[*Annals of Botany*, Vol. L, No. CXCVIII, April, 1936.]

Morris (3) showed some time ago that the activity of diastase changes during the daytime.

When making experiments on photoperiodism in the Institute of Horticultural Husbandry in 1932 and in the section of decorative plants and shrubs of the Academy of Municipal Economy in 1934, our object was to study the photoperiodic reaction and at the same time the photoperiodic induction, as well as to elucidate the connexion between the phenomena of photoperiodism and the state of the enzymatic system in the French bean (*haricot*), *chrysanthemum*, and *cineraria*.

#### METHOD OF WORK.

French bean (*Phaseolus vulgaris*). The experiment was made with two kinds of bean—*Rostovsky* (early kind) and *Jegalovsky* (late kind). The experiments were designed on the following scheme: (1) control (plants exposed the whole day), (2) plants having had during the whole of their vegetative period a twelve-hour day, from 6 a.m. till 6 p.m., (3) plants for twenty days after the appearance of the seedlings exposed to a shortened day of twelve hours, 6 a.m. till 6 p.m.<sup>1</sup>

The beans were sown in the open air on 25th June. The first seedlings came up on 1st July. The darkening of the plants was secured by a special movable box drawn over the plots of growing plants. During the vegetative period of the plants phenological observations were made. When flowering was almost over and the pods were beginning to form, samples were taken on two occasions, five plants of each variety, for evaluating the enzymatic activity.

The activity of the enzymes (catalase, amylase, saccharase) was determined in the fruit and in the leaves.

*Chrysanthemum (Chrysanthemum indicum)*. Two kinds of *chrysanthemum* have been investigated as to the enzymatic activity, namely, 'Olivier' and 'Queen Mary'.

The experiment was made according to the following scheme: (1) an entire day (control), (2) a twelve-hour day, (3) an eight-hour day. The shortened day (twelve- and eight-hour) was made with the following variations. The day was shortened: (a) from the moment the cuttings had taken root, (b) from the moment of the removal of the plants to a hot-bed, (c) from the moment of their transplantation in the open air, (d) a month after transplanting in the open air. The darkening of the plants was by means of a light-proof material. The cultivation of the *chrysanthemum* for the experiments was carried out in the usual way. The cuttings were made on 28th April. The enzymatic activity in the leaves was

<sup>1</sup> This length of exposure to short days has been chosen for the investigation of the photoperiodic induction as being most effective, according to the experiments of N. P. Rodnikov of the Timirjazev Agricultural Institute, and to the preliminary experiments of N. P. Krassinsky at the Horticultural Experimental Station in Moscow.

determined on four occasions in August. Leaves were taken from six to eight plants of each variety. All leaves (old as well as young) were taken for analysis.

The enzymatic activity of plants of the variety 'Olivier' was investigated in the following sets: (1) control, (2) darkened from the moment of transplantation to the open, (3) darkened from the moment of removal to the hotbed. The determination of the enzymatic activity of plants of the kind 'Queen Mary' was carried out with the following sets: (1) control, (2) darkened from the moment of removal to the hotbed, (3) darkened from the moment the cutting took root.

*Cineraria (Cineraria hybrida).* The cineraria was sown on 10th April, and on 20th April the experiment was carried out on the following scheme: (1) control (entire day), (2) twelve-hour day, (3) eight-hour day. Up to 31st May the plants were kept in a hothouse, after which they were removed to the hotbed. The hotbed was darkened by means of sheets of tarred paper put over the hotbed frames. The cineraria was cultivated by the methods of commercial gardening. Samples for determining enzymatic activity were taken on 25th June, 3rd July, and 10th Sept., from all the three varieties. The plants of 25th June, 3rd July (80-100), and those of 10th Sept. (seven plants), were from all the treatments. The enzymatic activity was determined in the leaves, all leaves being taken for analysis as in the chrysanthemum. In chrysanthemum and cineraria the activity of catalase, peroxidase, saccharase, and amylase was investigated.

*Determination of the activity of the enzymes.* The material used for analysis was reduced to small fragments in a meat-grinder in experiments with French beans and with a sharp knife in those with chrysanthemums and cineraria. The mass was carefully mixed and two samples of 25 gm. each were taken and thoroughly ground in a mortar for twenty minutes. The ground mass was put into a graduated retort of 250 c.c. with distilled water. Toluol was added and it was extracted for two hours with periodical shakings. In experiments with French beans the extract thus obtained was filtered; paper No. 598 being always used for filtration. For the method of work see Bach and Oparin (2), also Rona (18). In experiments with chrysanthemums and cinerarias the extract was treated for three minutes on a centrifuge at 1,000 r.p.m.

To obtain optimal conditions for the activity of the enzymes buffer mixtures of definite pH value were used: for catalase a phosphate buffer of pH about 7.0; for amylase and saccharase an acetate buffer of pH about 5.0.

The activity of catalase was determined by the method of Bach and Oparin (2). The difference in the number of c.c. of permanganate used for the titration of hydrogen peroxide in the test and the control served as a measure of the activity of the enzyme. The activity of the enzyme was calculated per 1 gm. of dry substance.



The activity of peroxidase was determined according to the method of Bach and Chodat. The activity of the enzyme was expressed in mg. of the obtained purpurogallin, calculated per 1 gm. of dry substance.

To determine the activity of amylase 10 c.c. of the filtrate or of the centrifugate were poured into Erlenmeyer retorts. 1 c.c. of 30 per cent. KOH was immediately poured in the control retorts, after which 20 c.c. of distilled water, 10 c.c. of acetate buffer ( $\text{pH} = 4.9$ ), 10 c.c. of a 2 per cent. solution of soluble starch, and a few drops of toluol were added to the test and control vessels. The vessels were placed for fifteen hours at  $30^{\circ}\text{C}$ . After this, 1 c.c. of a 30 per cent. solution of KOH was introduced into the test vessels to inactivate the enzyme. With beans 3 c.c. of the liquid were then taken for determining (according to the method of Issekutz) the quantity of the reducing sugars formed. The difference in quantity of the sugars in the test and in the control flasks, calculated in mg. per gm. of dry substance, served as a measure of the activity of the enzymes. Issekutz's method cannot serve for determining the absolute quantity of sugars in vegetable material, but it can be used for investigations with enzymes, as it is then only the difference between the sugar content in the test and in the control flasks that need be known.

In experiments with chrysanthemum and cineraria the determination of sugars was made by the method of Bertrand.

The activity of saccharase was determined in the same way as that of amylase, the only difference being the use of 2 per cent. solution of saccharose instead of starch.

At the same time as a determination of the enzymatic activity that of pH was also made. For determining pH 25–50 gm. of chrysanthemum and cineraria leaves were taken, reduced into small fragments, the juice of which had been pressed out by a handpress. In this juice the pH was determined electrometrically by a quinhydrone-calomel electrode.

#### EXPERIMENTAL PART.

*French bean.* The first seedlings of French beans of all variations came out at the same time on 1st July. From this date the plants were submitted to the various degrees of darkening. In the shortened day the plants developed faster than the control plants. Flowering of both varieties, *Rostovsky* and *Jegalovsky*, began seven to eight days earlier in the plants having a shortened day during the whole of the vegetative period, while in the plants having a shortened day for the first twenty days flowering was three to five days earlier than in the controls. During flowering measurements of plants were made, which showed that the plants with a shortened day at the beginning of their development surpassed the controls in height as well as in number of shoots. Later, at the end of flowering, the control plants outgrew the darkened ones.

When flowering was ending and the fruit was beginning to form, samples of plants were taken for the study of the enzymatic activity in all the variants. The activity of catalase, amylase, and saccharase in the fruit and in the leaves was separately determined by the above described method. The results of these determinations are given in Table I.

TABLE I.  
*Enzymatic Activity of the Bean (per 1 gm. of Dry Substance).*

Kind.	Variation.	Fruit.			Leaves.		
		Catalase.	Saccharase.	Amylase.	Catalase.	Saccharase.	Amylase.
'Rostovsky'	Plants darkened the whole of their vegetative period from 6 p.m. till 6 a.m.	82.0	148.0	1020.0	71.0	20.0	473.0
	Plants darkened for the first 20 days after the appearance of seedlings, from 6 p.m. till 6 a.m.	29.0	476.0	1135.0	—	89.0	497.0
	Plants not darkened (control)	20.5	2476.0	1157.0	52.0	134.0	541.0
'Jegalovsky'	Plants darkened the whole of their vegetative period from 6 p.m. till 6 a.m.	75.0	110.0	728.0	29.4	119.0	223.0
	Plants darkened for the first 20 days after the appearance of seedlings, from 6 p.m. till 6 a.m.	28.0	162.0	943.0	27.5	136.0	273.0
	Plants not darkened (control)	10.0	740.0	1287.0	19.4	187.0	—

An evaluation of the yield has shown that the fruit ripened more quickly on the darkened plots than on the controls having light the whole day (see Table II). A late kind of French bean, the 'Jegalovsky' was backward in its ripening by comparison with the 'Rostovsky' bean. As can be seen in Table II, the plots that had been darkened only twenty days occupy an intermediate position as to ripening, between the control and those partially darkened during the whole of the vegetative period. The pods of the 'control', when gathered, were somewhat larger than those of the plants submitted to darkening of different variations.

*Chrysanthemum.* The plants under different treatments developed very differently.

A shortening of the day reduced considerably the vegetative period (see Table III). The control plants of the kind 'Queen Mary', since the cuttings were put in very late, did not flower until winter.

The shortening of the day not only influenced considerably the time of flowering, but also the general development of the plant—the number of leaves, height, &c.

TABLE II.

*The Percentage of Ripe Pods in French Beans.*

Variety.	Control (entire day).	A 12-hour day, the whole of the vegetative period.	A 12-hour day during the first 20 days after the appearance of seedlings.
'Rostovsky'	28.6	77.9	59.5
'Jegalovsky'	5.9	37.3	19.6

TABLE III.

*Time of the Beginning of Flowering of Chrysanthemum.*

Kind.	Length of the day.	Period of shortening of the day.	Beginning of flowering.
'Olivier'	The 12-hour day	From the time of the plant's removal to a hotbed.	8th Aug.
"	"	From the time of its transplanting to the plot.	11th Sept.
"	Control (whole day)	—	19th Oct.
'Queen Mary'	The 8-hour day	From the time of the cutting's taking root.	7th Sept.
"	"	From the time of the plant's removal to a hotbed.	28th Sept.
"	Control (whole day)	—	Flowering not begun until winter.

The determinations of the enzymatic activity of the plants 'Olivier' were made on the 3rd and 13th August, in those of 'Queen Mary' on the 19th and 27th August.

The results of these determinations, as well as the data characterizing the state of the plants at the moment the samples for enzymatic activity were taken, are given in Table IV.

*Cineraria.* Measurements of cineraria, made twice during the vegetative period, have shown that the eight-hour day was somewhat behindhand in results as compared with the control and the twelve-hour plants, there being no difference between the two last.

TABLE IV.  
*Enzymatic Activity of the Chrysanthemum (per gm. of Dry Substance).*

Kind 'Olivier'.	Variation.	Dry substance.			pH.			Catalase.			Peroxydase.			Saccharase.			Amylase.			State of the plants.		
		3rd Aug.	13th Aug.	21-70	3rd Aug.	13th Aug.	6-13	3rd Aug.	13th Aug.	410-1	3rd Aug.	13th Aug.	371-9	3rd Aug.	13th Aug.	215-3	3rd Aug.	13th Aug.	67-3	35-7	3rd Aug.	13th Aug.
	Control.																					
A 12-hour day from the time of transplanting to the ground		21-69	22-74	6-28	401-1	465-4	128-2	135-9	241-6	174-7	59-9	31-3	With buds.									
		18-88	17-27	6-45	665-8	805-5	183-3	267-5	303-9	245-6	77-3	41-2	With big buds; flowering began the 8th Aug.									
Kind 'Queen Mary'.																						
	Control.	19th Aug.	27th Aug.	19th Aug.	380-3	391-2	253-9	208-9	540-0	385-3	283-1	131-5	Without buds	19th Aug.	27th Aug.							
An 8-hour day from the time of the plant's removal to a hotbed		12-19	12-56	6-74	664-7	720-2	275-6	260-4	691-1	617-9	266-6	110-3	Green buds									
		11-50	11-11	6-85	667-5	805-2	282-6	312-3	695-7	619-7	217-4	118-8	Incomplete flowering									

The enzymatic activity in cineraria plants was determined three times: the 25th June, the 3rd July, and the 10th September. The results of the determinations are shown in Table V.

TABLE V.

*Enzymatic Activity of Cineraria (per gm. of Dry Substance).*

Variation.	Dry substance.			pH.			Catalase.		
	25th June.	3rd July.	10th Sept.	25th June.	3rd July.	10th Sept.	25th June.	3rd July.	10th Sept.
Control	9.69	8.55	8.09	6.45	6.57	6.56	1444	1239	1247
The 12-hour day	8.05	8.74	7.90	6.44	6.71	6.65	1605	1173	1223
The 8-hour day	6.74	6.51	7.95	6.49	—	6.47	1004	1321	1252

Variation.	Peroxydase.			Saccharase.			Amylase.		
	25th June.	3rd July.	10th Sept.	25th June.	3rd July.	10th Sept.	25th June.	3rd July.	10th Sept.
Control	121.3	129.3	268.2	597.4	541.6	940.0	0 <sup>1</sup>	0 <sup>1</sup>	42.03
The 12-hour day	94.7	151.0	378.5	742.9	631.0	874.6	0 <sup>1</sup>	0 <sup>1</sup>	48.73
The 8-hour day	113.5	109.1	249.1	956.8	678.1	872.1	0 <sup>1</sup>	0 <sup>1</sup>	42.77

## DISCUSSION.

The shortening of the day in our experiments has caused an acceleration of development in both varieties of French bean, and this manifested itself by an acceleration of flowering and of fruiting and ripening of the pods. Thus both varieties proved to be short-day plants.

The shortening of the day led to marked changes in the state of the enzymatic system of the French bean. The activity of catalase in the leaves of the plants submitted to a short day during the entire vegetative period increased about 1.5 times, in the fruit of the Rostovsky varieties 4 times and in the Jegalovsky variety 7.5 times. Most important and interesting is the fact that darkening of the plants for twenty days after the seedlings had come up has caused a considerable increase in the activity of catalase both in the leaves and in the fruit, in comparison with the control.

A shortening of the day has considerably diminished the activity of saccharase and amylase in the fruit as well as in the leaves. The activity of saccharase both in leaves and in fruit of the two kinds was several times less; that of amylase has also been considerably weakened by the shortened day. Even a twenty-day darkening has considerably lowered the activity of saccharase and amylase by comparison with the control.

The chrysanthemum is a typical short-day plant. The shortening of the day has produced a sharp reduction of the vegetative period, and at the same time marked changes in the state of the enzymatic system (see Table IV). In both the varieties, 'Olivier' and 'Queen Mary', the activity

<sup>1</sup> Traces of the activity.

of catalase when the day was shortened was doubled, and that of peroxidase was 1.5 times as great.

The rise of activity in the above-mentioned oxidizing enzymes proceeds gradually; this is very apparent in 'Olivier', the catalase being, it seems, more sensitive to the shortening of the day.

The changes in the activity of saccharase and amylase due to a shortening of the day manifested themselves by an increase of 1.5-2 times in the saccharase in both varieties and by a small decrease in the activity of amylase.

Cineraria has been chosen for investigation as a long-day plant (see Laurie and Poesch (14).

A shortening of the day during four months until late autumn did not lead to any noticeable changes in the development of the plants. Nor have any regular definite changes appeared in the state of the enzymatic system (see Table V). In the beginning of September the activity of catalase, peroxidase, saccharase, and amylase was the same in all the treatments.

After comparing the data of the determinations of the enzymatic activity for the three cultures, the following conclusions can be drawn.

The changes in the activities of the oxidizing ferments of catalase and peroxidase are undoubtedly the most marked. They are quite regular and perfectly clear in the French bean and in the chrysanthemum. The absence of any change in the activity of catalase and peroxidase in cineraria (where no photoperiodic reaction has manifested itself) shows that the changes in the activity of catalase and peroxidase are not due merely to a shortening of the photoperiod but are definitely connected with the photoperiodic reaction, and in the French bean, with the photoperiodic induction, as well.

Our data confirm the view of Ljubimenko, expressed as far back as 1927 on the great importance of the oxidation-reduction processes in photoperiodism.

Theoretically an increased energy in the oxidizing system with a photoperiodic reaction in plants of a short day seems easy to understand. A shortened day has caused a shortening of the vegetative period in the French bean and in the chrysanthemum. Thus we have in our experiments the classical reaction of the plants to a short day, which was first discovered by Garner and Allard in the soy bean. Garner and Allard, in giving this plant a short day, accelerated the development of the plant, shortened its vegetative period, and caused it to ripen earlier. This is certainly connected with an increase of energy in the vital functions, which, in its turn must be connected with a greater power of the oxidizing processes and with a generally increased energy of the oxidizing system in the cell.

Determinations of the activity of saccharase and amylase in the French bean and in the chrysanthemum show that, when the day is shortened, regular changes in the above-mentioned enzymatic activities set in. Yet these changes proceed differently in the bean and in the chrysanthemum.

The direction they take in the activity of saccharase and amylase depends apparently upon the nature of the plant and its hydrolytic enzymes. There evidently does not exist a unique and general rule governing the changes in the activities of saccharase and amylase in short-day plants, although there is no doubt that the changes discussed are connected with the photoperiodic reaction; for the cineraria, notwithstanding a protracted shortening of the day, has manifested no regular changes in the activity of saccharase and amylase.

In so far as the hydrolysing enzymes direct the biochemical changes in plants, it seems that the absence of a general regularity in the changes of activity of these enzymes in a photoperiodic reaction with plants of a short day, harmonize well with the data of Arthur, Guthrie, and Newell (1) and of other authors' work, who have proved that carbohydrate-nitrogen metabolism does not depend on the length of the day and hence on photoperiodism.

In summing up the above we can state that the photoperiodic reaction, as well as the photoperiodic induction, are connected with considerable changes in the enzymatic system and that the changes in the activity of the oxidizing enzymes deserve special attention.

The question arises as to whether these changes in the enzymatic system are primary or secondary; it is at present difficult to give an answer to this. More extensive and detailed investigations are needed on the state of the enzymatic system of different plants belonging to short and long day types, at different periods of their development. It is necessary to obtain data of the activity of different enzymes and to connect them with the phases of development. Our investigation is but a beginning of work in this domain; yet we believe it can furnish some indications as to the nature of photoperiodism from the view-point of the state of the enzymatic system. We know that, although there have been many investigations on photoperiodism, the nature of the photoperiodic reaction remains obscure (compare Schick (19)).

Considering that the appearance of the photoperiodic reaction is to a certain extent connected with changes produced in the enzymatic system under the influence of the light conditions, we suppose that, in explaining photoperiodism the physico-chemical indicators of the cells must be taken into account. Therefore, according to our determinations, a shortening of the day influences the value of the oxidation-reduction potential, a fact which emphasizes the significance of the oxidation-reduction processes in photoperiodism. A shortening of the day changes also the value of pH (see Tables IV and V). Moreover, a change in the light factor apparently affects, according to our preliminary data, the state of the biocolloids. Most likely an important role is played (according to Went) in the photoperiodic reaction by the as yet hypothetical hormones of flowering.

Ljubimenko (15) puts forward the hypothesis of hormones which accelerate the formative processes as depending on the photoperiodic reaction and induction.

Our opinion is that a further study of photoperiodism must proceed on the lines of an extensive and many-sided investigation (biochemical, physico-chemical, and physical) of this phenomenon, and that a clearly defined and important place must be attributed to a study of the state of the enzymatic system and, first of all, of the oxidation-reduction enzymes.

#### SUMMARY.

1. A shortening of the day has caused sharp changes in the state of the enzymatic system of the bean (fruit and leaves) and of the chrysanthemum (leaves); with the cineraria the shortening of the day, during four months, has produced no definite changes whatever in the enzymatic system.

2. In the French bean and in the chrysanthemum the shortening of the day has called forth a very considerable and regular increase of activity of the oxidizing enzymes—catalase and peroxidase; the increase of activity of these enzymes was evidently gradual.

3. In cineraria (which according to Laurie and Poesch (14) is a long-day plant) a shortening of the day has not led to any definite regular changes in the activity of catalase and peroxidase; this agrees with the absence of any considerable difference in the general development of the plants submitted to a full or to a shortened day. Consequently, the increase in the activity of catalase and of peroxidase in the French bean and in the chrysanthemum is related to the photoperiodic reaction and the photoperiodic induction; it is not merely a result of changes in the duration of daylight.

4. A shortening of the day in the French bean and in the chrysanthemum has provoked regular changes in the activity of amylase and of saccharase; yet the direction of these changes is different for the French bean and for the chrysanthemum. Thus the changes in the activity of the hydrolysing enzymes under the influence of a shortened day depend apparently on the individual peculiarities of the plants and of their enzymatic systems.

5. Cineraria has manifested no definite changes in the activity of saccharase and amylase as the result of a shortened day.

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# Studies in Flower Structure.<sup>1</sup>

## II. On the Vascular Supply to the Nectary in *Ranunculus*.

BY

AGNES ARBER

With four Figures in the Text.

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### 1. INTRODUCTION.

FIVE years ago Mr. John Parkin showed me specimens of a curious form of *Ranunculus asiaticus* L., with which he had been concerned in the course of his work on the 'glossy' and 'mat' petals of the buttercups (8-11). These flowers aroused my interest in the vascular supply of the nectary in this genus, and on cutting serial sections of the petals of various species, certain peculiar and suggestive features were disclosed. On consulting the literature, however, it was found that neither M. Brouland, in his recent work on the floral anatomy of the family (5), nor M. Kumazawa, in his account of the structure of Japanese species of *Ranunculus* (7), had made any reference to the subject, while G. Hume Smith, in his study of the vascular anatomy of the Ranalian flower (12), though mentioning the

<sup>1</sup> This series forms part of the work undertaken with the aid of a grant from the Dixon Fund of the University of London; for the reference to the first instalment, see (4).

basal structure of the petal, did not distinguish between scale and nectary; his statements will be discussed later (pp. 311–313). As no other description of the vascular supply for the nectary has been met with (see Postscript, p. 319), it has seemed that some account of it ought to be put on record.

## 2. METHODS.

The vascular supply of the buttercup nectary is usually well preserved in flowers fixed in commercial methylated spirit. For examining the structure of the petal base as a solid object (Fig. 1, A, C, etc.), it was stained with alcoholic gentian violet, followed by eosin in oil of cloves to give translucency. There is a risk, in dealing in this way with a number of species, that a stray petal may get left behind, hidden by the dark staining fluid, and may then be mistaken for a petal belonging to some other species, which is passed through the stain later. For this reason I have found it best to carry entire flowers through the fluids, and not to detach the petals until the final xylol stage is reached. Preparations made in this way are useful for giving a general idea of the structure, but transverse microtome series are necessary if it is to be understood in detail. For these the modification of Fleming's triple stain devised by Sargent for anatomical work has been used: 1 per cent. Bismarck brown in 70 per cent. alcohol, followed by 0.3 per cent. aqueous gentian violet, differentiated with 2 per cent. aqueous orange G, followed by 1 per cent. orange G in 50 per cent. alcohol. Microtome series taken through whole buds or flowers are almost useless, because of the curvature of the petals and the angle at which they leave the receptacle; so it is best to cut off the basal regions of detached petals and embed them alone. For a complete treatment of the subject it would be necessary to keep the petals of each flower, and those occupying different positions within the same flower, separate in the paraffin, in order to see how the structural variation met with in the petals is related to the individuality of the plant or shoot, and to the topography of the flower. In the present slight sketch I have not attempted to do this, as it would have made it impossible to cover sufficient ground to give a general idea of the nectary supply in the genus.

Owing to the delicacy of the petals the nectary structure is not well preserved in herbarium specimens, so this study is based entirely on a few of the species, of which fresh or pickled material was available; and as the genus includes about 300 species (8), there may be many interesting variants which have been missed. In the following Section I have described my observations, leaving the general bearing of the work to be indicated briefly on pp. 316–318. The order in which the species are taken has been chosen merely for convenience in treating of the anatomy.

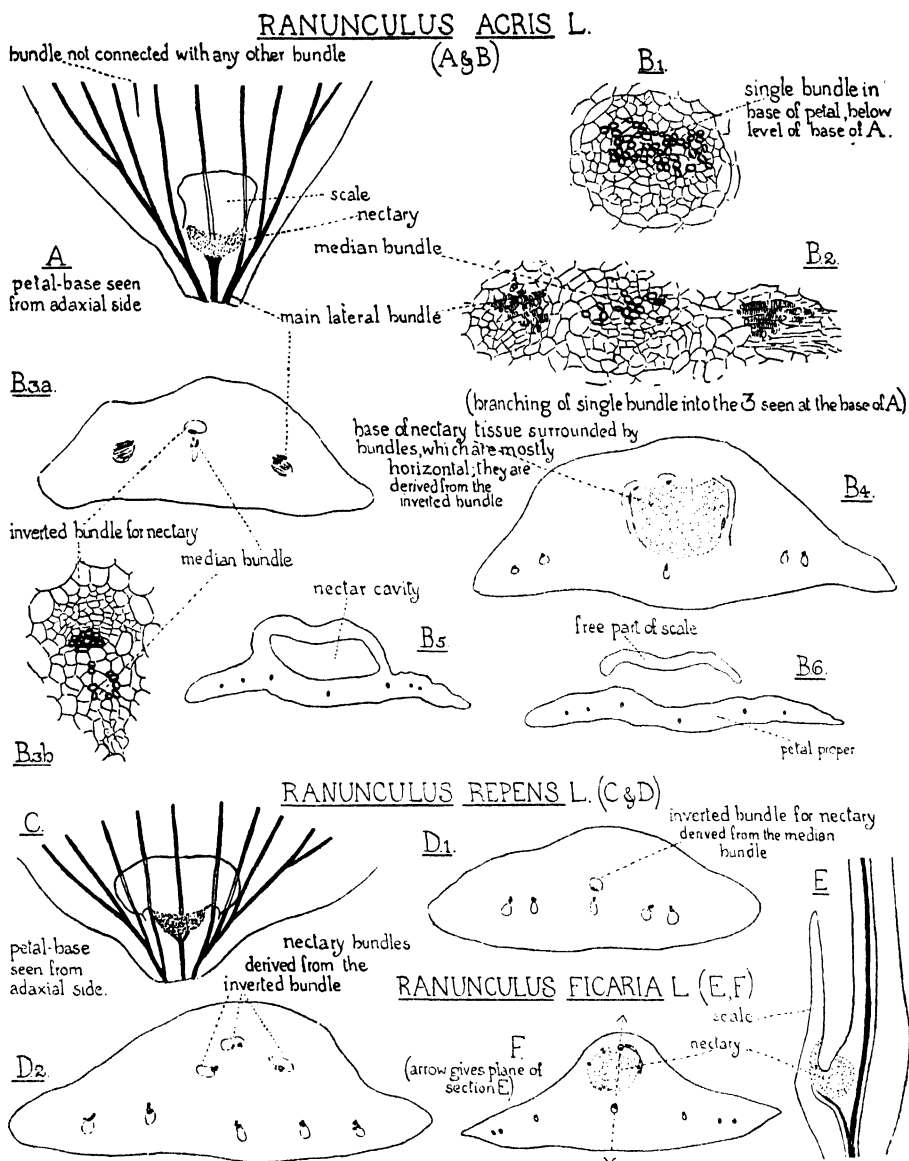


FIG. 1. Throughout the figures glandular tissue is dotted. In petal bases viewed as solid objects, the bundles are indicated in solid black except where they are seen faintly through the nectary scale. In sections, xylem is indicated in black and phloem in white. A, B, *Ranunculus acris* L. A, petal base viewed from adaxial side ( $\times 14$ ). B 1-B 6, sections from a transverse series from below upwards through a petal base; B 1, B 2, B 3 b ( $\times 193$  circa); B 3 a, B 4 ( $\times 47$ ); B 5, B 6 ( $\times 23$ ). B 3 b shows the median bundle in B 3 a, with its inverted branch, on a larger scale. C, D, *R. repens* L. C, petal viewed from adaxial side ( $\times 14$ ). D 1 and D 2, sections from a transverse series from below upwards through a petal base below the level of the nectary ( $\times 47$ ); lignified xylem elements indicated individually. E, F, *R. ficaria* L. E, radial longitudinal section ( $\times 23$ ) of a petal base, in the plane marked with an arrow in F (semi-diagrammatic reconstruction from longitudinal series). F, sections from a transverse series through a petal passing through the base of the nectary ( $\times 23$ ); E and F being from different petals are not consistent in proportions.

## 3. OBSERVATIONS.

*R. acris* L., Fig. 1, A, B, p. 307.

The general construction of the petal base is seen in Fig. 1, A; there the veins are 'blackened in', except where they are perceived faintly through the nectary scale. Figs. B 1—B 6 show transverse sections taken from a series passing from below upwards through a single petal, from the base to a level at which the nectary scale has become free from the petal proper. At the extreme base, below the lowest level shown in A, there is a single collateral bundle with lignified xylem above and phloem below (B 1), which almost at once divides into three. This division has just occurred in B 2, in which the lateral bundles are cut at an angle, as they diverge obliquely from the main bundle. The next change is that in the median bundle the phloem creeps round the xylem, until there is more of it on the adaxial than the abaxial face. The xylem also divides, and we reach the stage shown in B 3 a and B 3 b, in which the new bundle is completed; it has its xylem directed towards the xylem of the median bundle, from which it differs in the greater width of its phloem. This bundle we may distinguish as the *inverted bundle*. At a higher level it divides and gives rise to a slight weft of strands below the nectary tissue and surrounding its basal region; these strands include some lignified elements (B 4). B 5 passes through the nectar cavity above the level of the secretory tissue; the vascular supply of the nectary does not extend to this level. In B 6 the free non-vascular part of the nectary scale is shown. In this and in two other petals all the bundles for the nectary were derived from the median bundle, but, in a fourth petal, the two lateral veins also contributed to the nectary system.

*R. repens* L., Fig. 1, C, D, p. 307.

The petal base of this species shows a general similarity in external appearance (C) and in section (D 1, D 2) to *R. acris*. In two petals the entire nectary system was found to be derived from an inverted bundle arising from the median bundle (D 1), and subsequently branching (D 2). In D 1 and D 2 the lignified elements are indicated individually. In a third petal there was the slight variant that the median bundle, after producing the inverted bundle, gave off a small additional strand for the nectary system.

*R. Ficaria* L., Fig. 1, E, F, p. 307.

F shows in transverse section the nectary of *R. Ficaria*, and its associated strands, some of which have lignified elements. In three petals I found that the nectary was supplied from the median bundle only. In E an attempt is made to represent the petal base in radial longitudinal section. Even in this species, in which the structure is relatively simple, longitudinal sections do not give an adequate idea of the nectary system in

relation to the petal bundles, and such sections can be used only to indicate the general topography; in other species in which the nectary supply is more elaborately developed, a drawing of a radial longitudinal section may be, not only inadequate, but even actively misleading.

*R. bulbosus* L., Fig. 2, A, p. 310.

In two petals of *R. bulbosus*, of which serial sections were cut, the inverted bundle from the midrib gave all the nectary strands. The nectary supply is more fully developed than in the species hitherto considered. In A 1 *a* the position of the more or less horizontal web of strands below the gland tissue is indicated, and its structure is shown in greater detail in A 1 *b*. It includes some bundles with lignified xylem. From this web delicate strands run vertically in the marginal region of the nectary tissue on the abaxial side of the cavity; at the level of A 2 *a* and A 2 *b* they are unlignified. The free part of the scale is, as usual, non-vascular.

*R. caucasicus* Bieb. (cult. J. Parkin).

In this species serial sections were taken through three petals. In each of them the median bundle gave off an inverted bundle which was solely responsible for the nectary system.

*R. velutinus* Tenore, Fig. 2, C, D, p. 310 (cult. J. Parkin).

Serial sections were cut through three petals of this species; in two of them the median bundle and both the main laterals contributed branches to the nectary system, while in the third this system was supplied from the median bundle and one lateral only. The latter scheme is illustrated in the petal whose base is seen whole in C; the main bundle, and the lateral on the left, give off branches to the nectary, but the lateral on the right plays no part. In this species the nectary system is relatively well lignified. In the petal in which only one lateral contributed to this system the horizontal strands below the base of the nectary, and also the upward branches which surrounded the base of the nectary on the side towards the median bundle, were well lignified; at the base of the cavity seven lignified strands were seen on this side, but only one unlignified strand on the other side. In another petal drawn in D, in which both the laterals as well as the median bundle supplied the nectary, lignification extended on the side away from the median bundle to a level above the base of the cavity. The lignification of bundles *x* and *y* ceased just above the level of D, but both bundles continued as phloem strands, *x* to about the level at which the nectary scale became free, and *y* for a shorter distance.

*R. oreophytus* Delisle, Fig. 3, A, B, C, p. 312 (cult. J. Parkin).

In this species I have found a good deal of structural variation in different petals. A 1–A 3, and B 1–B 3, are from one petal. A 1–A 3 shows the

## RANUNCULUS BULBOSUS L (A)

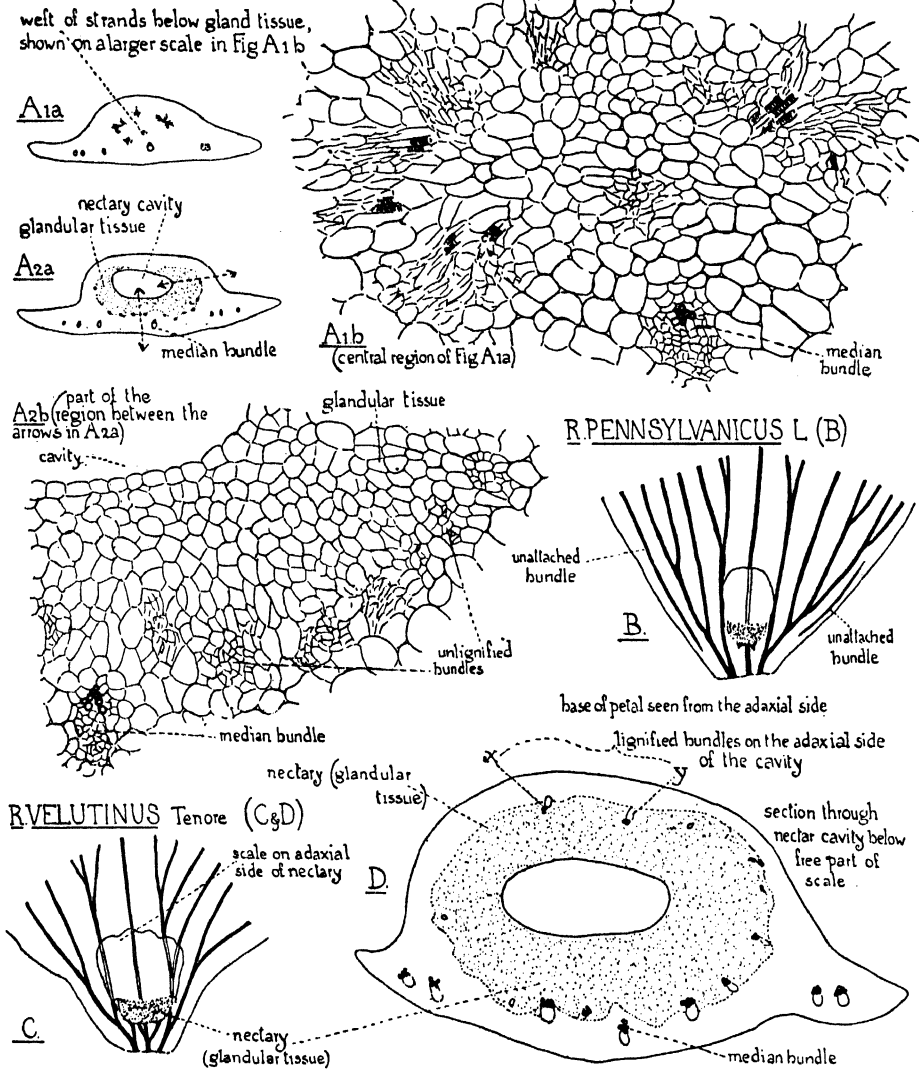


FIG. 2. A, *Ranunculus bulbosus* L. A1 a-A2 b, sections from a transverse series from below upwards through a petal base. A1 a, at base of nectary ( $\times 23$ ); A1 b, central region of A1 a ( $\times 193$  circa). A2 a, through nectary cavity ( $\times 23$ ); A2 b, the part of A2 a between the arrows ( $\times 193$  circa). B, *R. pennsylvanicus* L., base of petal viewed from the adaxial side ( $\times 14$ ). C, D, *R. velutinus* Tenore. C, petal base viewed from adaxial side ( $\times 14$ ). D, section from a transverse series through a petal to show the nectary cavity and associated strands ( $\times 77$  circa); lignified elements indicated individually.

median bundle giving off the inverted bundle, which in this petal is solely responsible for the nectary system; it supplies the glandular tissue richly with vascular strands which surround it and also penetrate into it. A remarkable feature of this particular petal is that one branch from the nectary system turns away from the main mass of secretory tissue, and supplies a subsidiary nectary (B 1, B 2). This second glandular mass lies somewhat to one side of the nectary scale (B 3). I found a second nectary in one other petal, but here it appeared to be supplied by a branch from one of the main laterals and not from the median bundle. Though it happens that a second nectary occurs in two of the only four petal bases of which I have complete series, I can see no sign of any additional nectary in several petals mounted whole, and I do not know whether the occurrence of extra glandular tissue is frequent or not. In the third petal base from which I have a complete series there was a single nectary, which was supplied by bundles from the median strand alone. In a fourth petal, in which, again, only one nectary was developed, one lateral as well as the median bundle contributed to the system for a single nectary. The web of strands below the glandular tissue in this petal is sketched in C 1, while in C 2 the nectary is cut through near the level of the base of the cavity, with a number of small, very delicate strands running in its margin. Some of these are so ill-defined that it is difficult to know whether to count them or not; I reckon the total number as approximately twenty-seven. About seven of those on the side towards the median bundle have one or more lignified elements, but none of those on the adaxial face of the nectary are lignified at this level. In another petal, at a level corresponding to the interval between C 1 and C 2, I noticed that one of the vertical branches from the plexus on the abaxial side (away from the midrib) possessed more lignified elements than the median bundle itself, though in the latter the lignification was stronger. Perhaps it is the high development of the vascular system for the nectary of this species which renders possible the production of extra glandular tissue.

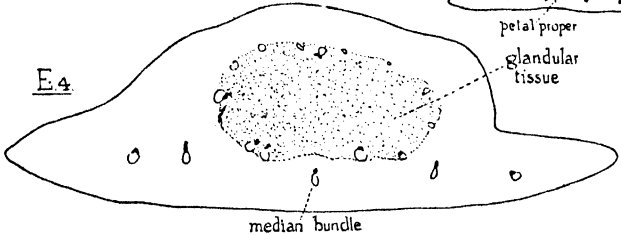
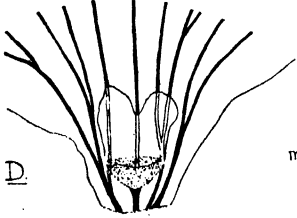
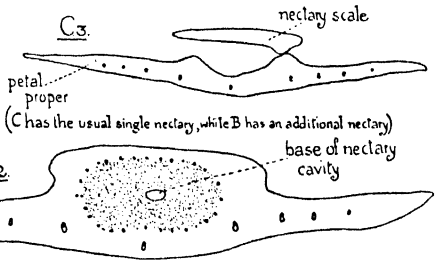
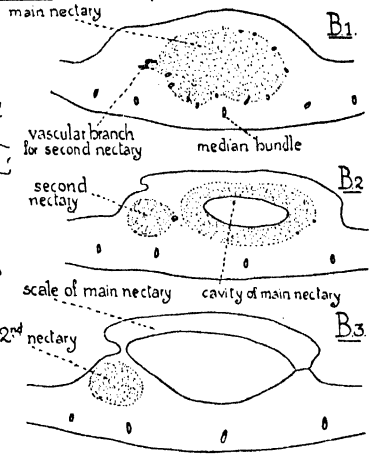
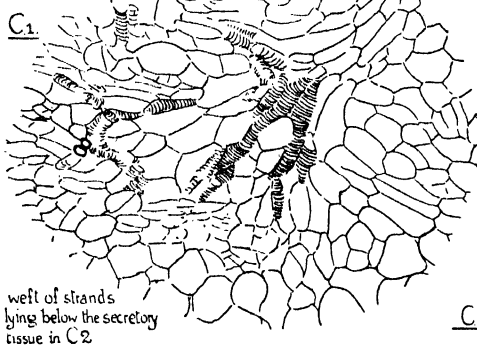
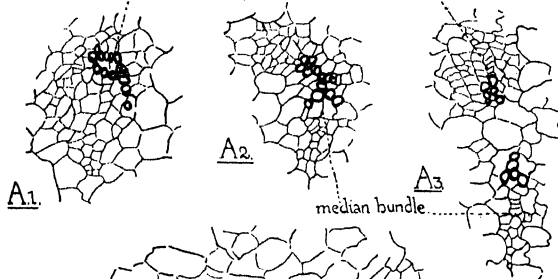
*R. septentrionalis* Poir., Fig. 3, D, E, p. 312 (coll. and fixed by C. A. Weatherby, Sunderland, Vt., U.S.A., June, 1935).

In this species, according to G. Hume Smith, 'the first branch of the median vein of the petal arises from the ventral surface of the vein and passes up into the small scale situated at the base of the petal' (12, p. 18). No mention is made of the nectary itself. This description and the accompanying diagram (12, Fig. 25, p. 16, which is of a stylized type, and does not show the nectary) suggest that there is a single bundle passing straight up into the scale. As I have not found this arrangement elsewhere in the genus, I was glad to have an opportunity of examining this species. It will be seen from Fig. 3, D, E 1-E 6, that I have been unable to confirm



# RANUNCULUS OREOPHYTUS Delisle (A,B,C)

A<sub>1</sub>-A<sub>3</sub>, median bundle of petal giving rise to inverted bundle



## R. SEPTENTRIONALIS Poir

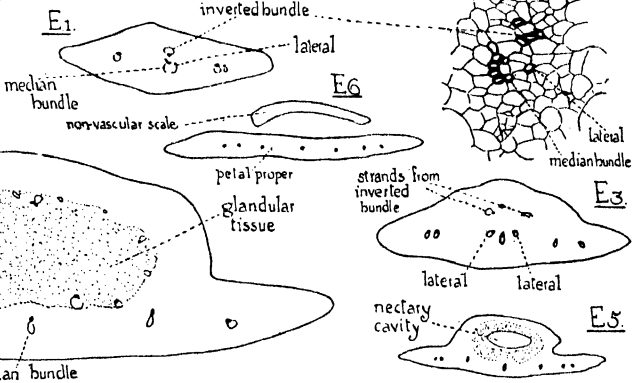


FIG. 3. A-C, *Ranunculus oreophytus* Delisle. A, B, sections from a transverse series, upwards from below, through one petal. A<sub>1</sub>-A<sub>3</sub>, origin of inverted branch from collateral median bundle ( $\times 193$  circa). B<sub>1</sub>-B<sub>3</sub>, median region of the petal ( $\times 23$ ); B<sub>1</sub>, through main nectary below cavity; B<sub>2</sub>, through main nectary in region of cavity; B<sub>3</sub> above glandular tissue of main nectary; B<sub>2</sub> and B<sub>3</sub> show second nectary. C<sub>1</sub>-C<sub>3</sub>, sections from a transverse series from below upwards through another petal base. C<sub>1</sub>, through the base of the nectar cavity ( $\times 23$ ); C<sub>3</sub>, through the free part of the scale ( $\times 14$ ). C<sub>1</sub> ( $\times 193$  circa) shows the weft of strands below the nectary at a lower level than C<sub>2</sub>. D and E, *R. septentrionalis* Poir. D, petal base viewed from adaxial side ( $\times 14$ ). E<sub>1</sub>-E<sub>6</sub>, sections from a transverse series from below upwards through a petal. E<sub>1</sub>, E<sub>3</sub> ( $\times 23$ ); E<sub>2</sub> ( $\times 193$  circa); E<sub>4</sub> ( $\times 47$ ); E<sub>5</sub>, E<sub>6</sub> ( $\times 14$ ). E<sub>2</sub> shows the median bundle in E<sub>1</sub>, and the two branches it is giving off for the nectary, on a larger scale.

G. Hume Smith's account, and that the structure, in the examples which I was able to study, falls into line with that of other buttercups. In five petals, of which I cut transverse series, no strand, lignified or unlignified, rises into the free part of the scale. Moreover no lignified bundle penetrates even into the lower part of the scale where it is fused marginally with the petal surface; but a few delicate unlignified strands may rise for a short distance into this attached basal region of the scale. The origin of the vascular supply for the nectary in this species varies considerably, and is not so simple and diagrammatic as in some of the other buttercups. In the series illustrated in E 1–E 5, the median bundle contributes three strands which give rise to the nectary supply; one of these seems to correspond to the inverted bundle of *R. acris* etc., while the other two are lateral. In another petal there was no individual inverted bundle, but the median of the three main bundles gave off two laterals, each of which divided, thus forming a group of four bundles as the basis of the nectary supply. In another petal there was again no single inverted bundle, but the bundle given off for the nectary branched immediately in the horizontal plane.

*R. pennsylvanicus* L., Fig. 2, B, p. 310 (cult. Cambridge Botanic Garden).

As a contrast to *R. velutinus* and *R. septentrionalis*, in which the vascular system for the nectary is highly developed, *R. pennsylvanicus* may be noticed, as a species in which this system is much reduced. In three petals indications were found that a non-lignified bundle for the nectary was derived from the median bundle, but in two others no connexion was traceable between the slightly developed and non-lignified vascular system of the nectary and any of the petal bundles. It will be noticed in Fig. 2, B, that in the petal proper there are two marginal bundles, which do not run for the whole length of the petal, and which have no connexion, basally, apically, or by branching, with any other vein; they may be called 'free' bundles. Among eight petals, stained and mounted whole, I found in four, one free non-marginal bundle; in one, one free marginal; in one, one free marginal and one free non-marginal; in one (Fig. 2, B), two free marginal; in one, three free non-marginal. It is suggestive to find this discontinuity in the general vascular system of the petal, in a species which also tends to discontinuity between the main veins of the petal and the strands for the nectary; but it is not possible to lay much stress on this point, as free bundles of a corresponding type may occur in the general petal system of *R. acris* (Fig. 1, A) and *R. repens*.

*R. gramineus* L., Fig. 4, A–F, p. 314 (cult. Cambridge Botanic Garden).

Unlike the buttercups hitherto considered, in which the nectar cavity is bounded by an adaxial scale on one side and the upper surface of the petal on the other, *R. gramineus* belongs to the type in which the nectary occurs at the base of a tubular structure, which on the side towards the

# RANUNCULUS GRAMINEUS L (A-F)

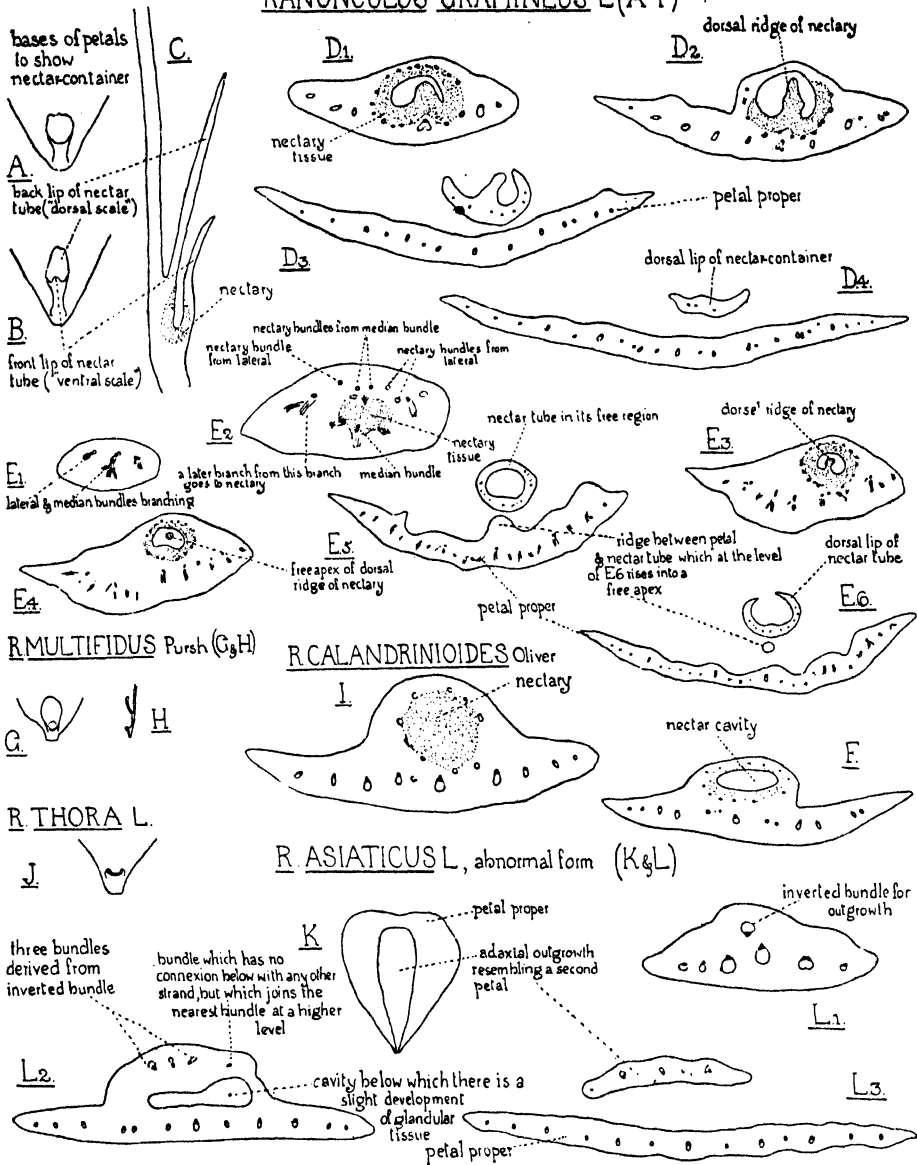


FIG. 4. A-F, *Ranunculus gramineus* L. A, B, bases of petals with honey tube viewed from adaxial side. In A, the length from base of petal to top of tube lip was just over 3 mm.; in B, 4 mm. C, radial longitudinal section of petal base, vascular tissue omitted ( $\times 14$ ). D 1-D 4, sections from a transverse series from below upwards through another petal base ( $\times 14$ , except E 2,  $\times 23$ ). E 1-E 6, sections from a transverse series from below upwards through another petal base ( $\times 14$ , except E 2,  $\times 23$ ). F, transverse section of petal passing through the nectar cavity ( $\times 23$ ). G, H, *R. multifidus* Pursh; G, petal base enlarged, viewed from adaxial side; length in this example from base of petal to tip of scale, 2.75 mm.; the scale is variable in size and form. I, *R. calandrinoides* Oliver, section from a transverse series through a petal base ( $\times 23$ ). J, *R. Thora* L., petal base viewed from adaxial side; in this example length from petal base to top of ridge, about 1 mm. K, L, *R. asiaticus* L., abnormal form, cult. Mr. G. P. Baker, 1930. K, duplex petal viewed from adaxial side ( $\times \frac{1}{2}$ ). L 1-L 3, sections from a transverse series through the base of a petal similar to K ( $\times 14$ ).

petal is continued upwards to form a free rim (the so-called 'dorsal scale'; see 8). Figs. A, B, C, show the external appearance and radial section of this nectar-container. There is a good deal of variation in its size and shape, but the figure given by Troll (14, Fig. 45, vii, p. 196) does not conform to the appearance of any of the flowers that I have examined. Some idea of the range of variation may be gained from Figs. D–F. In the series D the ventral lip of the nectar-container does not rise above the level at which the dorsal lip becomes free, so there is no free tubular region. There is a median ridge in the nectary tissue (D 1 and D 2) which is continued up into the free lip (D 3). In E, on the other hand, the ventral side of the lip is more developed, so that there is a free basal cylindrical region. The median ridge occurs here (E 3) as in D, and terminates in an isolated upward peg (E 4). A second peg-like outgrowth, which is not found in D, occurs between the back of the lip and the petal (E 5, E 6). In a third petal, whose nectary cavity is seen in section in F, there is no median ridge of nectary tissue, such as that seen in D and E. The vascular supply of the nectary in D, E, and F, is formed of components from the median bundle and the two main laterals (see E 2). There is so little lignification in the nectary bundles that their orientation cannot be determined.

*R. asiaticus* L., Fig. 4, K, L, p. 314 (cult. G. P. Baker, Sevenoaks).

Mr. Parkin tells me that in *R. asiaticus* 'in the wild form (dry material) the nectary is a basal shallow depression. In cultivated forms the nectary tends to disappear.' In May 1930 he kindly sent me four petals from a flower of a peculiar white form of this species, which he had received from Mr. G. P. Baker. These petals were duplex in character. An adaxial lobe, also white and looking like a second smaller petal, arose from the base of each (Fig. 4, K). In one case the length of the lobe was within 2 mm. of that of the petal proper. Sections showed that the bundles of this outgrowth had their xylems turned towards the xylems of the petal (L 1–L 3), the vascular scheme thus recalling the orientation of the main bundle for the nectary system in other buttercups. There was a slight but recognizable trace of glandular tissue at the base of the cavity between the petal and its outgrowth—so slight that it scarcely claimed any vascular supply. The small bundle to the extreme right of the inverted series in L 2 united at a higher level with the nearest bundle; basally it had no connexion with any other bundle, but it gave off a small branch which also ended blindly, and suggested a vestigial nectary supply. The meaning of the adaxial outgrowth, which is a curious feature in a species which does not normally possess a distinct ventral scale, will be considered on p. 317.

As an addendum to these observations it may be noted that serial sections of the petals of various species of *Ranunculus* often show—as might be expected—grains of pollen in the nectar-container.

## 4. COMPARISONS AND CONCLUSIONS.

(i) *The Source of the Vascular Supply to the Nectary in Ranunculus.*

The buttercups have been divided by Parkin (8) into two sections characterized by the nature of their petal surfaces and the forms of their nectaries—a relatively small group of 'mat-petalled' species, in which the nectary is either naked or associated with an outgrowth extending chiefly on the dorsal side (Fig. 4, A–C); and a much larger group of 'glossy-petalled' species in which the nectary is either naked (Fig. 4, J) or covered by an upgrowth on the ventral side (Fig. 1, A 1, etc.).<sup>1</sup>

In the species of the 'mat' type which I have examined (*R. gramineus* and *R. calandrinoides*), the vascular supply for the nectary is derived from the median bundle, and from either one or both of the two main laterals. It is so poorly lignified that the orientation of the bundles cannot be determined. The nectary structure is thus of less anatomical interest than in the members of the 'glossy' section of the genus, in which the bundles are in general better developed.

In the buttercups of the 'glossy' type illustrated in Figs. 1–3, the general scheme of structure can be understood from the sketches of petal bases seen whole. The nectary system is either derived from the median bundle alone (e.g. *R. repens*, Fig. 1, C), or the median bundle and one main lateral (e.g. *R. velutinus*, Fig. 2, C), or the median bundle and both main laterals (e.g. other petals of *R. velutinus*); or, on the other hand, the nectary system may be extremely reduced, and entirely, or almost entirely, isolated from the petal system (e.g. *R. pennsylvanicus*, Fig. 2, B).

(ii) *The Further History of the Vascular Supply to the Nectary in the 'Glossy' Buttercups.*

The most striking anatomical feature of the nectary supply in the 'glossy' buttercups is that, in general, its main bundle, which arises from the ventral surface of the median vein of the petal, is *inversely orientated* (e.g. *R. acris*, Fig. 1, B 3 b; *R. repens*, Fig. 1, D 1; *R. Ficaria*, Fig. 1, E; *R. oreophytus*, Fig. 3, A 3). This bundle subdivides and forms (often with the addition of components from the main laterals) a web of strands below the glandular tissue (Fig. 2, A 1 a and A 1 b; Fig. 3, C 1) from which arises a radial arrangement of delicate fibrils surrounding the glandular tissue (Fig. 2, A 2 a and A 2 b; Fig. 3, C 2; Fig. 4, I). The occurrence of an extra nectary in certain petals of *R. oreophytus* (Fig. 3, B 1–B 3) may perhaps be correlated with the unusual richness of the vascular supply to the nectary in this species.

The inverted bundle and its associates are used up so completely

<sup>1</sup> *R. multifidus* Pursch is an exception, for here a nectary with a dorsal scale (Fig. 4, G, H) is associated with glossiness; see 10.

in producing the nectary supply system that I have never seen lignified bundles in the free part of the scale, though they may rise above the level of the base of the cavity (e.g. *R. velutinus*, bundles *x* and *y*, Fig. 2, D).

(iii) *The Interpretation of the Scale in the 'Glossy' Buttercups.*

The fact that the nectary scale in the 'glossy' buttercups is typically non-vascular in its free region obscures the problem of its interpretation; but some light is thrown upon it by a study of an abnormal form of *R. asiaticus* (one of the 'mat' buttercups) described on pp. 315-316. In this form the products of the inverted bundle (Fig. 4, L 1), instead of being used for the nectary, which was almost non-existent, were found to pass straight into an adaxial petal lobe (Fig. 4, K), which thus acquired a vascular system orientated inversely as regards the petal proper (Fig. 4, L 3). This adaxial lobe may, perhaps, be interpreted as corresponding to the ventral scale of the 'glossy' buttercups, but in a hypertrophied form. If this correspondence is valid, the non-vascular character of the ventral scale in normal buttercups must be explained on the ground that the inverted bundle, which is really the trace for the scale, is diverted, and broken up to supply the nectary, so that there is no vascular tissue left on the ventral side vigorous enough to pass right up into the scale. If this theory is sound we must regard the ventral scales of the 'glossy' buttercups as falling into the category of enations from the ventral surfaces of leaf members, which follow the 'law of laminar inversion' (15). Čelakovský, with his usual insight, suggested long ago that the basal scale of the buttercup petal should be regarded as *facing* the petal (6). He came to this conclusion without knowledge of the anatomy, which is now shown to reinforce his view. In a later paper I hope to pursue the comparison of the nectary scales of *Ranunculus* with structures in related types, and also with enations, such as the coronas of certain *Amaryllids*, which are known to be supplied by inverted bundles.

(iv) *The Petal and Nectar-container of Ranunculus, Considered from the 'Gestalt' Standpoint.*

Troll's book on the flower (14) has made us aware of the importance of correspondences between structural systems which are not homologous, but which nevertheless belong to the same 'Gestalt' type. In this sense I think we may compare the relation of the ventral nectary scale of *Ranunculus* (i) to its petal, and (ii) to its nectary, with the relation of an axillant leaf (i) to its parent axis, and (ii) to its axillary bud. The nectary resembles an axillary bud in the more or less radial plan of its vascular system, but in contrast to an axillary bud its growth is inhibited. Now on the principle of growth-compensation, whose importance has recently been emphasized

by Thompson (18), this limitation may well lead to the production of further marginal upgrowths of various forms, giving the tube or 'dorsal scale' of the 'mat' buttercups.

(v) *The Predominance of Phloem in Nectary Supply Bundles.*

I have noticed elsewhere that in the vascular supply of floral nectaries in the Cruciferae, Fumarioideae, and Hypecoideae, phloem predominates (1, pp. 30-3; 2, pp. 342-3; 3, pp. 164-5). This is also true of *Ranunculus*. The relatively large amount of phloem in the inverted bundle, which will give the nectary supply of *R. acris*, is seen in Fig. 1, B 3 b. In all the buttercups which I have examined, the individual nectary strands, even if lignified at the base, terminate upwards in phloem alone. This seems to indicate that the carbohydrates in solution, which the gland uses for the manufacture of nectar, must travel—at least in the final stages of their journey—*via* the phloem.

So far as can be determined from microtome sections stained for general anatomy, the phloem tissue of the nectary supply consists entirely of cells of cambiform type, which often have elongated nuclei. A more complete histological study might, however, reveal greater complexity.

## 5. SUMMARY.

In this paper the vascular supply to the petal nectary is described for the following species of *Ranunculus*:—*acris* L.; *repens* L.; *Ficaria* L.; *bulbosus* L.; *caucasicus* Bieb.; *velutinus* Tenore; *oreophytus* Delisle; *septentrionalis* Poir.; *pennsylvanicus* L.; *gramineus* L.; *calandrinoides* Oliver; and an abnormal form of *asiaticus* L.

It is shown that the nectary supply, in which phloem markedly predominates, is derived from the median bundle of the petal, often with the addition of components from the two main laterals. In the most typical of the 'glossy' buttercups the nectary branch, which arises ventrally from the median bundle, is inversely orientated, with its xylem facing the xylem of the median bundle. This inverted bundle may be regarded as the trace for the nectary scale, but since this trace is used up in supplying the nectary, the scale is left non-vascular in its free region. The idea that the scale is, despite its non-vascular character, an enation which follows the law of laminar inversion is confirmed by a study of a peculiar form of *R. asiaticus*, in which a reduced nectary is associated with an outgrowth resembling a hypertrophied ventral scale supplied by bundles of inverse orientation.

For material I am indebted to the kindness of Professor Edgar Anderson of the Missouri Botanic Garden, to Mr. G. P. Baker of Sevenoaks, to Mr. C. A. Weatherby, Curator of the Gray Herbarium, Cambridge, Massachusetts, and to the Curator of the Cambridge Botanic Garden. I must

also express my gratitude to Mr. Parkin, not only for supplying me with flowers of the species of *Ranunculus* which he has under cultivation, but also for his constant help during the progress of this study and for criticism of the manuscript.

#### POSTSCRIPT.

Since this paper was written I have found that I had overlooked two references which should have been included. The anatomy of the petal base in *Ranunculus acris* L. was described by G. Bonnier 'Les Nectaires. Ann. Sci. Nat., Bot., VI, viii, p. 102, Pl. 2, Figs. 16, 17, 1879'. E. J. Salisbury, 'On the Morphology and Ecology of *Ranunculus parviflorus* L., Ann. Bot., xlv, pp. 539-78, 1931' gives a photograph of a radial longitudinal section of the petal of this species, and writes, 'The petal is supplied by a single trace which passes to the base of the nectary, where it expands beneath the secretory tissue (Pl. XVIII, Fig. 4); from this expansion five slender strands are given off which pass into the limb of the petal.' (I have not found an example of this type of structure among the species which I have examined.)

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# The Preparation of 'Three-colour' Strips for Transpiration Measurements.

BY

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With one Figure in the Text.

SOME twenty years ago Livingston and Shreve (1) described an improved technique for using cobalt chloride paper in transpiration measurements. To replace the older method of timing the change of colour of unstandardized cobalt paper from 'blue' to 'pink', a procedure introducing manifest uncertainties and errors, they devised oblong strips each comprising a small square of cobalt paper fixed between two standard tints, one dark blue and one light. The 'three-colour strip' so formed, after having been warmed until the cobalt paper in the middle section was of a deeper tint than the 'dark' standard, was transferred to the leaf and held in position by a glass slip, through which one could observe and accurately time the march of the change of colour from the moment it matched the dark standard until that at which it matched the light one.

Some five years later, requiring relatively large numbers of the strips to meet the large wastage entailed in practical teaching, and being dissatisfied with the colour-matching obtained by using Livingston's method of tinting the paper with Prussian blue, the writer modified the procedure somewhat, so as to make a better matching and to produce the strips more easily.

These *ad hoc* modifications, of course, involve no new principle, nor do they supersede Livingston and Shreve's rigidly standardized methods for research purposes; they are useful for the teacher of practical physiology, however, and repeated requests for their details seem to justify their publication.

*Preparation of standards.* In the making of tinted papers, four points have to be taken into account—the nature of the paper, the evenness of wetting, the time-factor in the adsorption of the dye, and the possible progressive dilution of the dye solution by the paper. In addition to these,

[Annals of Botany, Vol. L. No. CXCVIII. April, 1936.]

variation may occur in different samples of the dye. In making, for instance, the tinted papers according to Livingston and Shreve's instructions, not only is it difficult, as they observe, to repeat either the cobalt chloride and ferric chloride solutions, but also a difference in texture of the paper, or a trace of foreign substance, such, for example, as size or other loading, will give distinct differences in the colour obtained from the same solution of dye. It is well, therefore, to adopt, and rigidly to follow, a definite technique, to use always the same type of paper, and to set aside one's own sample of dye, a gramme of which will last for many years.

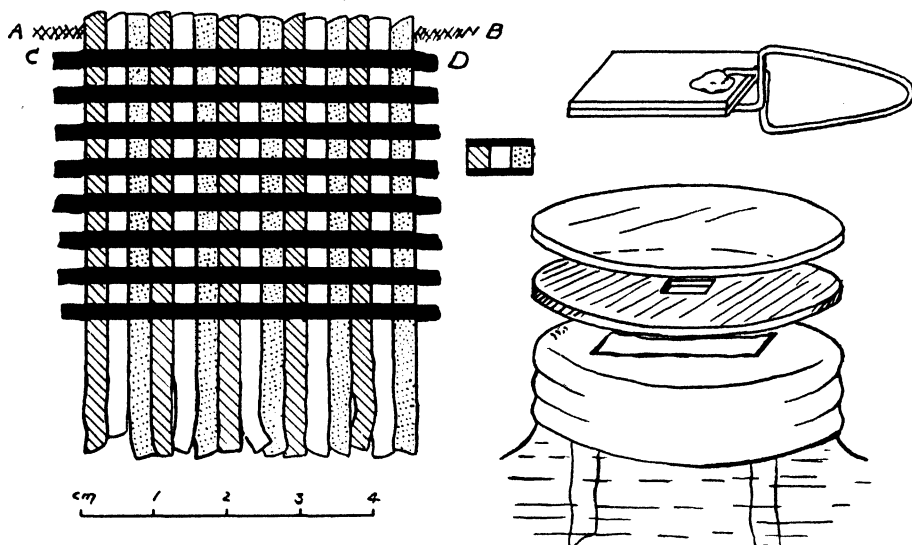
The standard cobalt chloride solution used by the writer contains 150 gm. of the salt in a litre of water. The salt is, of course, hygroscopic, but a five per cent. deviation of strength does not appear to change appreciably the depth of colour.

The blue standard solutions are dilutions made from a stock solution of methylene blue, containing 1 gm. in a litre of water, and are rapidly made by diluting this stock solution. The dark blue standard solution is one-eighth full strength, and the light blue, one-thirty-second. For correction of the light blue tint a very dilute solution of eosin is used, as described below. The solution, which should be changed after treating three discs, is contained in a large Petri dish of 17 cm. diameter. A similar dish containing water, a photographic rubber squeegee roller, a quantity of blotting-paper, stopwatch, forceps, and drawing-pins are also required. The paper employed is Whatman's No. 1 filter paper, in discs 15 cm. in diameter.

The procedure is as follows: Lift a disc of paper in the forceps and immerse quickly and evenly 'edge-on' into the water in the first Petri dish. Leave to soak for one minute, remove, and squeegee fairly firmly between blotting-paper sheets. Immerse in the appropriate dye, or in the cobalt solution, for one minute, remove, and squeegee. Pin up to dry. The light blue standard will be found to be slightly greenish compared with the corresponding tone in the cobalt paper. This is rectified by subjecting it after drying to a further immersion for one minute in a solution of eosin of strength 1 in 10,000, squeegeeing, and drying.

The strips are now assembled thus: With a sharp knife, or a photographic trimmer, cut each of the coloured discs into narrow lengths 3 mm. wide, and from a piece of thin black photographic masking paper cut about two dozen similar narrow pieces approximately 10 cm. long and 2 mm. wide. Upon a piece of stout white paper lay, using the nozzle of the tube-container, a thin smear of seccotine, but in quantity sufficient to ensure that it will not dry for several minutes (AB, on p. 323). Next, assemble the coloured paper strips in the correct order as shown, ensuring that they lie touching one another. Do not stick the lower ends down, but leave them free. Now apply seccotine to one of the narrow black strips with the

nozzle of the tube, wipe longitudinally with a finger-tip to distribute the adhesive evenly, and lift the strip, still adhering to the finger, into the transverse position CD. When accurately in position, press it firmly down with a sharp straight-edge, and repeat the operation until the ends of the coloured strips are reached, adjusting them when necessary by their free ends.



When the seccotine has set, cut each of the black cross-strips along the middle with sharp scissors, and, lastly, separate the individual three-colour strips from one another.

With a stock of coloured papers at hand, it takes about an hour to make a hundred strips. They are not truly permanent, as are those described by Livingston and Shreve, and will ultimately fade, but they keep for at least two years in the dark.

The clips for holding the three-colour strips upon the leaf have the form of blunt forceps with glass ends (see Figure) between which the leaf and the strip are held. These clips are conveniently made from two pieces of glass, 2.5 cm. long by 1 cm. wide, cut from an ordinary microscope slide and cemented with molten shellac or de Khotinsky cement to No. 16 gauge hard brass wire bent to the shape shown.

For a standard water-surface, a metal screw-cap 4-ounce bottle, such as are sold containing sweets, serves well. In the metal cap two parallel slots 1 cm. by 1 mm., are cut about 2 cm. apart, and through these a strip of filter paper 1 cm. wide is passed, the ends dipping into water in the bottle, as shown. Upon the damp surface so formed a disc of celluloid 1 mm. thick with a central perforation 5 mm. square is placed, and upon

that a flat glass disc. This corresponds to the porous-pot apparatus of Livingston and Shreve.

The method of use of these three-colour strips is widely known, and is, of course, exhaustively dealt with in Livingston and Shreve's original paper.

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# The Influence of Certain Fungi on the Sporulation of *Melanospora destruens* Shear and of Some Other Ascomycetes.

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With Plate V.

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### 1. HISTORICAL.

THE first record of the action of one organism in stimulating the sporulation of another is that of Molliard (9) in 1903. He noticed that a species of *Ascobolus*, growing on carrot, produced apothecia when the cultures were contaminated by a bacterium, but did not fruit in pure cultures.

A similar effect was noted by Sartory (14, 15, 16). He claimed that the presence of bacteria was necessary for the sporulation of a yeast and for the production of the perithecia of a species of *Aspergillus*. The stimulating effect of the particular bacterium was shown more clearly on certain media than on others.

[Annals of Botany, Vol. L. No. CXCVIII. April, 1936.]

In 1909, Heald and Pool (5) published the first record of the stimulatory effect of one fungus on the sporulation of another. They worked with *Melanospora pampeane* Speg., which gave only a scanty white mycelium when grown in pure culture, but which produced perithecia in abundance when grown in mixed culture with *Fusarium moniliforme*. Similar stimulatory effects were produced by *F. culmorum*, *Basisporium gallarum* and an undetermined species of *Fusarium*, but not by another undetermined species of *Fusarium*. The mycelial growth produced by *B. gallarum* or *F. moniliforme* was removed, and the media were sterilized at 110° C. and inoculated with *M. pampeane*. Perithecia were formed within a few days on media prepared from young cultures of *Basisporium* or *Fusarium*, but growth of the *Melanospora* was inhibited in media prepared from older cultures. Heald and Pool concluded that certain chemical compounds are necessary for the development of the perithecia of *M. pampeane*, and that these compounds are regularly produced by *B. gallarum* and *F. moniliforme* and, possibly, by other fungi.

Zeller and Schmitz (20), working with mixed cultures, showed that the usual effect of one organism on another was a mutual inhibition of growth, before or after actual contact of the mycelia took place. Only in a few cases did one fungus colony grow over another, and very rarely was any actual stimulation of growth observed. In some cases, however, the sporulation of one organism was stimulated by the presence of another. Thus there was an increase in the number and size of conidiophore heads of *Aspergillus Sydowi* when in contact with *Merulius pinastri* and of *A. niger* when in contact with *A. glaucus*. Zeller and Schmitz state that they found no definite relation between the acidity produced by these fungi and their ability to inhibit or stimulate the growth and sporulation of other fungi.

Porter (12), experimenting with a number of bacteria and fungi, found evidence of growth changes and antagonistic action occurring in mixed cultures. He observed that the action of one organism on another stimulated sporulation along the line of contact between the colonies. In many cases malformation also occurred. With a species of *Helminthosporium* he was able to obtain somewhat similar effects, on both sporulation and malformation, by the use of certain poisons.

McCormick (8) found that no perithecia were formed in monoconidial cultures of *Thielavia basicola* Zopf, whereas when ascospores were used as inocula perithecia were formed. Mono-conidial cultures, however, readily produced perithecia when grown in mixed cultures with *Cladosporium fulvum*, *Aspergillus umbrosus*, *A. glaucus*, *Eurotium amstelodami* and *Fusicladium pirinum*.

In contrast to the results of Heald and Pool (l.c.), McCormick was unable to demonstrate any activity in fungal extracts which had been

heated to 110° C. for twenty minutes, but extracts of active fungi sterilized by passage through a Berkefeld filter did stimulate perithecial formation.

Wilson (19) stated that, in the presence of certain fungi, *Venturia inaequalis* formed perithecia more abundantly in a zone near the periphery of the contaminating colony. A mycelial extract of *Penicillium* sp. gave a similar result. Autoclaving diminished the activity of the extract.

The effects of colonies of *P. glaucum* and of extracts of this fungus on the growth and sporulation of a number of other fungi were described by Sibilia (17). The *Penicillium* extract markedly stimulated conidial production in *Microcera coccophila*, but the latter did not produce conidia when grown in mixed culture with *P. glaucum*. Vegetative growth was stimulated in a number of other fungi.

All the papers mentioned above describe the stimulatory effect of one organism on the sporulation of another. Nadson and Jolkevitch (11), however, describe the antagonism between *Spicaria purpurogenes* and *Saccharomyces cerevisiae* and state that, when these two organisms are grown together, the yeast is killed and the formation of conidia by *Spicaria* is completely inhibited.

Gwynne-Vaughan (4), in a review of the question of sex and nutrition in the fungi, cited the case of *Humaria granulata*, a heterothallic form in which, although both strains produced well-grown female organs, ascogenous hyphae were not formed unless both + and - strains were present. The behaviour of this fungus led Gwynne-Vaughan to seek an explanation of heterothallism which was not based on a difference in sex but on a difference of metabolism of the one strain brought about by the presence of the second strain.

Wineland (18), finding that two strains of *F. moniliforme*, neither of which produced the perfect stage in pure culture, did so when mated in the same culture tube, suggested that these facts might be interpreted as a case of normal heterothallism, or that alternatively they might be accounted for by the chemical stimulation of one strain by the other.

Working with strains of *Glomerella*, Egerton (3) showed that, although both so-called + and - strains of this fungus could form fertile perithecia in pure culture, they did so more readily when in contact with one another. This suggests a form of nutritive heterothallism or chemical stimulation.

Moreau and Moruzi (10) claimed that neither the union of nuclei nor the contact of hyphae of + and - strains of *Neurospora sitophila* was necessary for the production of fertile perithecia. They grew two strains of this fungus (neither of which produced fertile perithecia in pure culture, but which did so when grown together) in opposite ends of a U-tube containing culture medium. After some time fertile perithecia were produced at one end of the tube. Since it was claimed that no mycelium was present in the central part of the tube, these authors concluded that the production



of perithecia was due to the diffusion of some substance through the culture medium from one colony to the other.<sup>1</sup>

Das Gupta (2) reported that two infertile saltants of *Cytosporina ludibunda* produced pycnidia when the mycelia were allowed to intermingle. These saltants were strikingly unlike in morphological character. Das Gupta thought it unlikely that the difference between them was sexual in nature, and concluded that the formation of pycnidia when the two strains intermingled was a form of 'nutritive heterothallism'.

## 2. EXPERIMENTAL METHODS.

A strain of *Melanospora destruens* Shear, which was isolated from a diseased apple in the Plant Pathological Laboratory of the Imperial College of Science,<sup>2</sup> showed a striking behaviour in that, while it formed perithecia in pure culture on certain media, it did so more freely when certain contaminating organisms were also present.

Stock cultures of the strain of *M. destruens* were maintained in tubes of 1.5 per cent. malt agar at laboratory temperature. In order to reduce the risk of the cultures becoming sterile, perithecia were included in the inoculum at each successive reculturing.

Petri-dish cultures, incubated at 25° C. were used for the study of perithecial formation. The methods of experiment were generally of the type usual in mycological work. Special methods were occasionally used and will be described in the appropriate places in the text.

It was necessary to devise a method of measuring perithecial frequency on any given plate. The perithecia of *M. destruens* have light coloured walls but black spores, so that when mature they appear black and are readily visible to the naked eye. In any plate which had been inoculated at the centre the density of perithecia varied somewhat from one part to another, usually increasing from the centre outwards. Plates of standard size (11 cm. diameter) were always used. These were divided into ten equal sectors by drawing ten equidistant radii on the bottom of the plate, the first radius being drawn at random. The number of perithecia per microscopic field (at a magnification of 60) was determined for four points on each radius, these points being at arbitrary distances from the centre. Thus the figure given for perithecial frequency for each plate is the average of forty determinations. In each experiment the number of plates was replicated at least five, and often ten, times.

The number of perithecia per unit area on any particular medium

<sup>1</sup> An attempt was made to repeat this experiment during the present work. This was unsuccessful, since even with a depth of 5 cm. of medium in the U-tube the hyphae grew through from one side to the other.

<sup>2</sup> Isolated by Dr. A. S. Horne and identified by Mr. Mason of the Imperial Mycological Institute.

varied with the depth of medium in the plate. Accordingly, throughout the investigation, each plate of standard size received approximately 50 c.c., so that variations in perithecial frequency due to differences in depth of medium were avoided.

As time went on, sporulation of the *Melanospora* became progressively less free, so that the values for perithecial frequency on a standard medium are not comparable throughout this paper. Values given in any one Table are strictly comparable.

### 3. FACTORS INFLUENCING SPORULATION IN PURE CULTURES OF *M. DESTRUENS.*

#### (a) *Nature of the Nutrient Medium.*

In testing the effects of various contaminating organisms on sporulation of *M. destruens* it was desirable to avoid media which were either entirely unsuitable for the formation of perithecia, or on which they formed in such abundance that any further increase in their number would be difficult to detect. A suitable medium for this purpose would be one on which fruiting occurred rather sparingly.

TABLE I.

Medium.	Perithecial frequency.
1. 3 % Malt	8.5
2. Coons' solution	2.8
3. Glucose 0.5 %, peptone 0.35 %	6.8
4. Glucose 1 %, peptone 0.2 %	5.2
5. Maltose 0.5 %, peptone 0.35 %	3.8
6. Glucose 0.5 %, potassium nitrate 0.35 %	2.4
7. Glucose 0.5 %, potassium nitrate 0.2 %	2.2
8. Glucose 0.5 %, asparagin 0.35 %	2.1
9. Glucose 0.5 %, asparagin 0.2 %	0.4
10. Glucose 0.5 %, ammonium nitrate 0.35 %	0
11. Glucose 0.5 %, ammonium tartrate 0.35 %	0

The number of perithecia per unit area, produced in fourteen days on a variety of media, is illustrated in Table I. The synthetic media 3—10 contained the following basal salt mixture:— $\text{KH}_2\text{PO}_4$ , 0.125—0.175 per cent.;  $\text{MgSO}_4$ , 0.075 per cent., with 1.5 per cent. agar.

The medium 6 (which will afterwards be referred to as (Medium A), on which moderate perithecial formation took place was chosen for further work. The formula of Medium A is: glucose, 5 gm.;  $\text{KNO}_3$ , 3.5 gm.;  $\text{KH}_2\text{PO}_4$ , 1.75 gm.;  $\text{MgSO}_4$ , 0.75 gm.; agar, 15 gm.; water, 1 litre.

Table I shows that the medium most favourable to perithecial formation among those tested was malt agar. One of the glucose peptone media was almost as good. Of the sources of nitrogen tested peptone was the best and salts of ammonia the worst. These results were amply confirmed by further experiments.

The number of perithecia formed on the full Medium A was compared with the number formed on a series of media from which a single constituent was lacking. This experiment was repeated several times, but variation in perithecial frequencies between individual plates of the same batch was sufficiently great seriously to diminish the significance of some of the results. The following results, however, were significant. The absence of phosphate materially reduced the amount of growth of the *Melanospora* and no perithecia were formed. Growth was less seriously reduced by the absence of nitrogen,

TABLE II.

Medium used.	Perithecial frequency.		
	6 days after inoculation.	14 days after inoculation.	27 days after inoculation.
A	0	0.22	2.4
0.6 A	0	1.3	2.6
0.2 A	0.6	1.8	2.0
Plain agar	0	0.06	0.4
A (0.6 G)	0	1.4	2.4
A (0.2 G)	1.7	2.26	2.3
A (0 G)	0.32	0.4	0.6
A (0.6 N)	0	0.2	2.3
A (0.2 N)	0	0.2	2.1
A (0 N)	0	0	0
A (0.6 P)	0	0.2	2.2
A (0.2 P)	0	0.18	2.3
A (0 P)	0	0	0

but sporulation was almost completely inhibited, a few scattered perithecia being formed about seven weeks after inoculation of the plates. The absence of magnesium sulphate did not significantly decrease either growth or perithecial frequency. Fruiting was actually hastened, by about four days, by absence of glucose, but growth was poor, and after three weeks there were normally more perithecia on Medium A than on that medium lacking glucose.

The *Melanospora* was grown on Medium A and on dilutions of that medium to 0.6 and 0.2 of the original strength (given as 0.6 A and 0.2 A in Table II) and on plain agar. It was also grown on a series of modifications of Medium A in which the concentration of glucose was reduced to 0.6 and 0.2 of that in the standard medium, the other constituents remaining unaltered [given as A (0.6 G) and A (0.2 G) in the Table] and on Medium A lacking glucose [A (0 G)]. Media in which the concentration of potassium nitrate and potassium dihydrogen phosphate were similarly reduced to 0.6 and 0.2 of that in the standard medium, or were lacking, were also used [given as A (0.6 N), A (0.2 N), A (0 N), A (0.6 P), A (0.2 P), A (0 P) in the Table]. The results of this experiment are given in Table II, the figures referring to perithecial frequency.

The results obtained by growing the fungus on a series of dilutions of Medium A and on a series of media in which the glucose content was varied were thus very similar. Dilution of the medium to 0.6 of its original strength or reduction of the glucose content to 0.6 of that in Medium A both led to an earlier production of perithecia. A similar reduction of the amount of nitrate or phosphate did not hasten fruiting, reduction of either of these to 0.2 of their original concentration having no significant effect. It is thus suggested that the stimulatory effects on sporulation of diluting the standard medium are largely due to the reduction in glucose content.

These results were confirmed by further experiments, and the fact was established that the actual concentrations of glucose and nitrogen present had a greater effect on sporulation than did the ratio of these, since with the same C/N ratio, widely different numbers of perithecia were formed. A similar statement was made by Robinson (13) for apothecial formation in *Pyronema confluens*.

The hydrogen-ion concentration of the medium had no marked effect on perithecial frequency within wide limits. Malic acid or sodium bicarbonate was added in varying quantities to the normal Medium A (pH 6.2) to give a range of pH from 3.5 to 8.6. All the media on which the *Melanospora* developed became slightly more acid during the experiment. Over the range of initial pH 4.8 to 7.6 there was no marked difference, either in mycelial development or perithecial formation. Outside these limits mycelial growth was reduced considerably, and only a few scattered perithecia were formed.

Krause (6) also found that, within wide limits, pH had no influence on the fruiting of seven species of Hypocreaceae, including a species of *Melanospora*.

#### (b) *Transference from One Medium to Another.*

Leonian (7), who made a detailed study of the factors promoting pycnidium formation in the Sphaeropsidales, stated that sudden transference from a concentrated to a dilute medium led to increased sporulation in some species, but had the opposite effect in others. He worked chiefly with liquid cultures.

In the present investigation the method adopted for transferring the fungus from one medium to another was to cut out a segment of agar medium (of standard size) in advance of a growing colony, and to replace it by a medium of different concentration. Petri dishes of Medium A were inoculated at the centre with *M. destruens*, and segments of the medium were cut out and replaced by media of strength 0.75 A, 0.5 A, 0.25 A, 0.1 A, 0.01 A or plain agar. Perithecia developed at the periphery of the segment, where a more dilute medium had been inserted, and this

zone of perithecia slowly spread inwards towards the centre. A typical culture plate from this series is illustrated in Pl. V, Fig. 3. Sporulation was greatest when the medium was replaced by 0.1 A, 0.01 A, or plain agar, and diminished with increasing concentration of the replacing medium. The perithecia began to form in the replaced segment within ten days from the time of inoculation, but later were scattered over the whole plate.

Transference of the fungus from Medium A to a weaker medium thus stimulated an earlier production of perithecia. This result is comparable with Claussen's well-known experiment with *P. confluens* (1).

(c) *Temperature.*

The rate of growth of *M. destruens* rose to a distinct optimum at a temperature of approximately 25° C. The optimum temperature for sporulation was also in the neighbourhood of 25° C., but the number of perithecia formed was remarkably uniform over the range 15–35° C.

(d) *Light.*

The effect of light was tested by exposing plates on the laboratory bench side by side with others wrapped in black paper. There was no significant difference in the number of perithecia formed, but those produced in continuous darkness were approximately 50 per cent. smaller.

(e) *Mechanical Barrier to Growth.*

When *M. destruens* was grown on a standard-sized plate of Medium A, perithecia developed first at the edge of the plate; and, at a later stage, were usually less numerous at the centre, than towards the edges (see Pl. V, Fig. 2). To test whether sporulation was stimulated by the presence of a mechanical barrier to growth, the fungus was grown on plates 4, 7, 9, 11, 14, and 20 cm. diameter. Perithecia were produced in all after two weeks. They arose first at the margin of the colonies, and extended towards the centre. In the smallest plate the growing margin of the culture reached the edge in four days, but no perithecia were formed until ten days later. In the largest plate they were visible on the fourteenth day, although the growing colony only extended part way across the plate. Sporulation was thus not induced by contact with the edge of the plate, but presumably first took place in the young parts of a colony of a suitable age.

Attempts to stimulate sporulation by cutting or rubbing the mycelium were unsuccessful.

Robinson (l.c.) was able to stimulate the formation of the apothecia of *P. confluens* by the use of various chemical checks to growth. Attempts to stimulate sporulation of the *Melanospora* by similar methods failed.

#### 4. THE INFLUENCE OF CERTAIN FUNGAL COLONIES ON THE FRUITING OF *M. DESTRUENS* AND OF CERTAIN OTHER ASCOMYCETES.

It has already been stated that *M. destruens* produced perithecia more readily in the presence of certain other organisms than in pure culture. Thirty species of fungi, together with two unidentified species of bacteria were tested in this connexion. Plates of Medium A were inoculated with the *Melanospora*, and with one of the other organisms, the two inocula being placed about 4 cm. apart.

Some of the organisms used as contaminants, viz. *Fusarium fructigenum*, *F. bulbigenum*, *Penicillium* sp., *Botrytis cinerea*, *Monilia fructigena*, and two unidentified species of bacteria caused a distinct ring of perithecia to be formed round their margins. Pl. V, Figs. 4 and 5 illustrate this type of effect.

With a second group of contaminants, *Sclerotinia trifoliorum*, *Gloeosporium fructigenum*, *Helminthosporium* sp., *Hormodendron* sp., and *Stagonospora Curtisii*, there was a definite zone of clear medium between the two colonies, across which neither fungus was able to pass. Perithecia were formed along the margins of the *Melanospora* colony in a distinct ring. Pl. V, Figs. 6, 7, and 8 show this type of behaviour.

The hyphae of a third type of contaminant, viz. *Phytophthora erythroseptica*, *Rhizopus nigricans*, *Absidia glauca*, *Blakeslea trispora*, *Sphaeropsis Malorum*, *Colletotrichum linicolum*, *Fusarium culmorum*, *Botrytis Allii*, and *Ascochyta Pisi*, intermingled with those of the *Melanospora* colony, the perithecia of the latter being formed on or below the surface of the contaminant colony. (See Pl. V, Fig. 9).

The fungi mentioned above caused an earlier production of perithecia by *M. destruens* when planted on plates of Medium A at the same time as the *Melanospora* was planted. Others, however, showed no stimulatory effect under these conditions. Certain fungi of this type, viz. *Mucor hiemalis* + and - strains, *Cunninghamella elegans* and *Pythium de Baryanum*, caused the formation of a distinct ring of perithecia round their margins if planted two to three days after the *Melanospora*. Others, viz. *Penicillium* sp., *Pestalozzia Hartigii*, *Rhizoctonia Solani*, *Sclerotinia cinerea*, and *Trichoderma* sp. produced a similar effect if planted one to two days before the *Melanospora*. Some of the organisms listed above as stimulating sporulation when planted simultaneously with *M. destruens*, failed to produce this effect if planted at a different time. Thus there is seen to be a very delicate balance between the *Melanospora* and the 'contaminant' which is upset if either be allowed to develop sufficiently rapidly to prevent normal growth of the other. If the contaminant used be either a rapidly growing organism, such as *M. hiemalis* or *C. elegans*, or be given sufficient start before the plate is inoculated with *M. destruens*, the latter is unable

to develop normally, and formation of perithecia is checked or actually inhibited. If, however, the contaminating organism be slow growing, or if the plate be inoculated with *Melanospora* some days before inoculation with the contaminant, then growth of the latter is insufficient to have any significant effect on the former.

Two of the fungi tested, viz. *Sporodinia grandis* and *Basidiobolus ranarum* produced negligible growth on Medium A. These also stimulated fruiting of *M. destruens* if plates of weak malt agar were used.

The effects of some of these fungi on certain other Ascomycetes were also investigated. *Fusarium fructigenum* and *Botrytis cinerea* were selected as suitable contaminants, and their effects were tested on eight species of Ascomycetes, viz. *Sordaria fimicola*, *Sordaria* sp., *Philocopra* sp., *Rosellinia necatrix*, *Chaetomium cochlioides*, *Ascobolus denudatus*, *A. viridulis* and *A. Leveillei*. Plates of Medium A were inoculated at the same time with the two fungi. The effects of the contaminants were not the same in every case, three different types of response being shown.

It has already been recorded that the presence of a colony of *F. fructigenum* or *B. cinerea* caused the formation of a distinct ring of perithecia by *M. destruens*. A similar response was shown by *Sordaria* sp., *S. fimicola*, *A. denudatus*, *A. viridulis*, and *A. Leveillei*. For example, perithecia of the *Sordaria* sp. were not formed in pure culture on Medium A until twenty-six days after inoculation, but a distinct ring of perithecia was formed round a colony of *F. fructigenum* six days after inoculation.

*R. necatrix* and *Philocopra* sp. fruited in both control and 'contaminated' plates. There was no concentration of perithecia round the edges of the contaminant colony, but they were formed earlier, and in greater numbers in the presence of the latter than in pure culture.

The strain of *C. cochlioides* used formed perithecia with equal rapidity, and in approximately equal quantity in both contaminated and pure cultures. This fungus was then tested in mixed culture with *M. destruens* when the sporulation of the latter was strikingly stimulated.

##### 5. THE EFFECTS OF PREVIOUSLY 'STALED' MEDIA.<sup>1</sup>

*Botrytis cinerea*, *Fusarium fructigenum*, *Gloeosporium fructigenum*, *Sclerotinia trifoliorum*, *Monilia fructigena*, *Cunninghamella elegans*, *Penicillium* sp. and *Helminthosporium* sp. were grown in conical flasks containing Medium A without agar. In the first few experiments the liquid was decanted off after ten to fourteen days, and a strong agar solution was added to it so that the mixture contained 1.5 per cent. agar and set when cool. Segments were cut from plates of Medium A, and were replaced by

<sup>1</sup> In this paper a 'staled' medium means one in which a fungus has grown, irrespective of whether it has reached the stage at which growth is checked.

the agar medium prepared from the 'staled' liquid. The plates were then inoculated at the centre with *M. destruens*. In some cases the *Melanospora* was planted four days before the replacement of the segment took place. These experiments were in general vitiated by the fact that spores of the staling agent were carried over with the staled medium. The effects produced were, however, very distinct, and where the *Melanospora* was planted some days before the replacement of the segment perithecia were produced earlier than could be accounted for by the development of colonies from the spores carried over in the medium.

Tests showed that the effects of the staled media were not significantly reduced by autoclaving for fifteen minutes at 115° C. In the later experiments, therefore, the staled liquid was strained through muslin, powdered agar was added, and the medium was autoclaved. In some cases tests were made by the replaced segment method, in others plates were poured with the staled medium and inoculated at the centre with *M. destruens*. The latter method had the advantage that quantitative estimates of perithecial frequency could be made by the method described above.

Certain of the fungi which it was desired to test did not grow well in liquid Medium A. These fungi were grown on plates of agar Medium A and, after a suitable interval, the medium was melted, strained, and reautoclaved. These two methods of preparation of staled media produced comparable results.

(a) *Replaced Segment Method.*

Sporulation of *M. destruens* was stimulated when the replaced segment consisted of medium staled by any of the fungi used in these experiments. If the staled medium were prepared from a young culture of any of the fungi tested the resulting effect was similar to that produced by replacing a portion of Medium A with a weaker medium. See p. 331 and Pl. V, Fig. 3.) In this case fruiting began at the outer margin of the inserted segment and progressed inwards. If, however, the inserted medium were prepared from older cultures, the perithecia were formed in a zone along the line of junction of the two media (Pl. V, Fig. 10). This zone then spread outwards or, in extreme cases, growth of the *Melanospora* was checked and perithecia were limited to the zone of junction of the media.

Similar results were obtained by growing the *Melanospora* in a small Petri dish containing Medium A, placed inside a larger one containing a staled medium, after the manner of Claussen's experiment (l.c.).

(b) *The Use of Whole Plates of Staled Medium.*

When plates poured with a staled medium prepared from fairly young cultures of any of the eight fungi listed above (p. 334) were inoculated with *M. destruens*, perithecia were formed more rapidly and more abundantly



than on Medium A. The intensity of this effect varied with the fungus used as staling agent.

Medium A on which *M. destruens* had been growing was autoclaved and reinoculated with the fungus. A slight, but significant, increase in perithecial frequency was observed. This increase was greater when the original cultures had already fruited before the medium was reautoclaved than when younger cultures were used.

TABLE III.

Days after inoculation.	Medium A.	Media staled by <i>B. cinerea</i> .		
		7 days.	18 days.	28 days.
6	0	0	1.0	0.3
13	0	2.2	2.3	0.7
21	2.2	3.0	3.4	0.7

TABLE IV.

Medium used.	Perithecial frequency.	
	6 days after inoculation.	20 days after inoculation.
A	0	0 <sup>1</sup>
A <sub>B</sub> × 4	4.2	5.0
A <sub>B</sub> × 3	2.2	3.9
A <sub>B</sub> × 2	1.8	3.5
A <sub>B</sub>	1.0	2.0
A <sub>B</sub> ÷ 2	0.8	0.9
A <sub>B</sub> ÷ 10	0	0.5
A <sub>B</sub> ÷ 30	0	0
A <sub>F</sub> × 3	1.0	2.0
A <sub>F</sub> × 2	0.6	1.3
A <sub>F</sub>	0.5	1.0
A <sub>F</sub> ÷ 2	0.3	0.8
A <sub>F</sub> ÷ 10	0	0.3
A <sub>F</sub> ÷ 30	0	0

The fruiting of the eight species of Ascomycetes listed above (p. 334), with the exception of *Chaetomium*, was quicker and more dense on plates of staled medium prepared in the usual manner on Medium A. *Chaetomium* fruited freely on fresh or staled medium.

The age of the culture from which the staled medium was prepared influenced the amount of stimulation of perithecial production by *M. destruens*. In a typical experiment flask cultures of *B. cinerea* in liquid Medium A were set up and allowed to grow at laboratory temperature for 7, 18, and 28 days respectively. The staled medium was then prepared in the usual way. The results, which are set out in Table III show that media prepared from 7 or 18 days old cultures of *Botrytis* had a stimulatory effect on the formation of the perithecia of *M. destruens*, but that those prepared from old cultures reduced the final number produced. Similar results were obtained with other staling agents. Accordingly staled media were always prepared from fortnight old cultures, unless otherwise stated.

Sporulation of the species of *Sordaria* and *Rosellinia* was even more readily checked in staled media from old cultures than that of *Melanospora*.

The effects of concentration or dilution of the staled media were investigated. The liquid in which the staling agent had been growing was strained and either concentrated over a water bath or diluted with distilled water to give a range of concentrations. The results of a typical experiment with media staled by *B. cinerea* or *F. fructigenum* are given in Table IV and show that, within the limits of the experiment, the amount of stimulation of perithecial production runs parallel with the concentration of the staled medium. In the table  $A_B$  means Medium A staled by *Botrytis*,  $A_F$  means Medium A staled by *Fusarium*.

A culture solution which has been staled by the growth of a fungus differs from the original in containing substances produced by the fungus and in having a reduced amount of the food substances which were present at the beginning. It was of interest, therefore, to determine how far the stimulating effect of the staled solutions, described above, was effected by the complete or partial restoration of some or all of the original constituents. Table V illustrates such an experiment. By the symbol  $A_B + 0.2 A$  is meant that to the solution staled by *Botrytis* the constituents of Medium A were added in such an amount that, apart from any residue present in the staled medium, the concentration in the mixture was one-fifth that of the fresh medium; and similarly for the other mixtures included in the Table. The amount of glucose present in the staled medium  $A_B$  was estimated as 0.22 per cent., i.e. approximately two-fifths of that originally present.

The results set out in the first six lines of the Table show that the addition of all the constituents of Medium A increases the stimulatory effect up to a point, but that further additions sharply diminish it. Nevertheless the Medium  $A_B + A$ , which obviously contains more of each food substance than does Medium A, is more favourable than the latter for perithecial development.

The addition of glucose alone to the staled medium gives results closely similar to those obtained when all the constituents are added. The same trend is shown where nitrate or acid-phosphate is added, but with these a higher concentration, in relation to that originally present, is necessary for the suppression of the stimulatory effect. The general result is, therefore, that the stimulating effect of a staled medium on perithecial production is removed by the addition of a sufficient amount of any of the food substances, but that a total concentration which is unfavourable in the fresh medium does not suppress perithecial formation in a staled one.

Similar results were obtained when medium staled by *F. fructigenum* was used. The effects of adding glucose to various other staled media were

<sup>1</sup> At the time of this experiment *M. destruens* did not form perithecia on Medium A until about 28 days after inoculation.

tested by the replaced segment method and were in good agreement with those described above for media staled by *B. cinerea*.

The foregoing results suggest that the staled liquids contained certain substances which stimulated perithecial formation and certain others consisting partly at least of unused residues of the original medium, which acted

TABLE V.

Medium used.	Perithecial frequency.	
	6 days after inoculation.	20 days after inoculation.
A	0	0
A <sub>B</sub>	1.0	2.0
A <sub>B</sub> +0.2 A	1.75	2.9
A <sub>B</sub> +0.4 A	0.85	2.5
A <sub>B</sub> +A	0	1.0
A <sub>B</sub> +2 A	0	0
A <sub>B</sub> +0.2 G	1.9	2.65
A <sub>B</sub> +0.4 G	0.75	2.4
A <sub>B</sub> +G	0	1.3
A <sub>B</sub> +2 G	0	0
A <sub>B</sub> +0.2 N	1.5	2.5
A <sub>B</sub> +0.4 N	1.85	2.6
A <sub>B</sub> +N	2.7	2.9
A <sub>B</sub> +2 N	0	0
A <sub>B</sub> +0.2 P	0.8	2.3
A <sub>B</sub> +0.4 P	0.85	2.6
A <sub>B</sub> +P	1.4	3.0
A <sub>B</sub> +2 P	0	0

A<sub>B</sub> = medium staled by *Botrytis*; G, N, and P represent respectively the amount of glucose, KNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>, present in medium A.

TABLE VI.

Staling agent.	Days after inoculation.	Medium A.	Perithecial frequency.			
			Staled medium.	Ether-insoluble fraction.	Ether-soluble fraction.	Re-combined fractions.
<i>Helminthosporium</i> sp.	9	0	1.7	6.5	0.1	—
(staled 14 days)	12	0	1.8	12.5	0.1	—
<i>Cunninghamella elegans</i>	8	0	0.6	10.7	0.7	0.5
(staled 14 days)	15	2.0	2.9	12.3	1.1	2.8
<i>Fusarium fructigenum</i>	15	2.1	No growth	9.0	No growth	No growth
(staled 21 days)						
<i>Botrytis cinerea</i>	11	0	2.3	4.0	0.5	2.4
(staled 14 days)						
<i>Penicillium</i> sp.	11	0	1.75	2.1	0.75	—
(staled 14 days)						

in the opposite direction. Attempts were made to separate some of these substances by fractionation with ether. For this purpose liquid media, staled by certain fungi, were shaken up in acid solution with two volumes of ether. After separation of the layers and removal of ether, agar was added to each fraction and the usual tests were made by both the standard methods. Illustrations of the results obtained, by the segment method are

given in Pl. V, Figs. 10, 11, and 12. Perithecial frequencies obtained in a typical experiment with the whole-plate method are summarized in Table VI.

Clearly the ether-insoluble fraction gave a much more pronounced stimulatory effect than did the original staled medium. The effect of the ether-soluble fraction was, in general, comparable with that of dilution of the medium, viz. an earlier production of perithecia, but a lower final perithecial frequency. The increased activity of the ether-insoluble part was not due to the effects of traces of ether, since if the two fractions were recombined the resulting effects were similar to those produced by the use of the original staled medium.

Sporulation of the species of *Sordaria* and *Rosellinia* was also greater in the ether-insoluble fraction than in the staled medium, and the ether-insoluble fraction in some instances stimulated sporulation even when the staled medium itself inhibited growth and sporulation.

## 6. DISCUSSION.

The effect of one organism or of its products upon the sporulation of another may be considered to arise from a variety of causes, such as mechanical or chemical interference with growth, change in the reaction or in the osmotic pressure of the medium, reduction in concentration of food substances or production of substances which induce or favour sporulation. The discussion of the factors responsible will now be taken up in the light of the foregoing results.

When *M. destruens* is grown in Petri dishes of about the ordinary size used in culture work, sporulation begins some little time after the fungal colony has reached the edge of the plate. Mechanical interference with growth might thus be suggested as an inducing factor. There is, however, no connexion, as the experiments with large plates quoted above (p. 332) have shown. Under any given set of conditions sporulation begins after a certain time interval, independently of whether the culture has reached the edge of the plate or not. Moreover mechanical interference cannot explain the increased sporulation on certain staled media.

The association of sporulation with a chemical check to growth is suggested by such appearances as are illustrated in Pl. V, Fig. 6, and Pl. V, Figs. 7 and 8, where a zone of clear medium separates the 'contaminant' colony from that of the *Melanospora*, perithecia being formed on the margin of the latter. Here again the two effects are not necessarily correlated. Experiments described above have clearly shown that sporulation may occur on media prepared from young or, in certain instances, from old cultures of the staling organism. Whereas media prepared from old cultures reduce the rate of growth of the *Melanospora*, those prepared from younger cultures do not, and usually give a growth rate of the fungus

greater than that on the unaltered medium. The only effect of growth-retarding or inhibiting fungal products on sporulation is to determine where, under certain experimental conditions, the formation of the perithecia occurs (as in Pl. V, Fig. 10), or, when such substances are present in large amount, to suppress perithecial formation altogether (see Table VI).

The acidity of the various preparations which influence sporulation is, within wide limits, of no importance. Adjustment of various staled liquids to the pH of the original medium does not reduce the stimulating effect, and the fungus sporulates freely over a wide range of initial pH.

Changes in concentration of medium have a distinct effect upon sporulation, but not in any direct relation to osmotic pressure. Thus increased concentration of the fresh medium, above a certain point, suppresses sporulation (Table II), while increased concentration of certain staled media increases it (Table IV). The addition of various food substances, and especially of glucose, to staled media reduces or suppresses their spore-promoting quality (Table V). Conversely dilution of a fresh medium (Table II), or transference from one medium to a more dilute one, as when the culture of *Melanospora* is allowed to grow from Medium A on to a segment of diluted agar (Pl. V, Fig. 3), stimulates sporulation. It is clear from these results that, while the concentrations of the various media have a marked effect on sporulation, the effect is not simply due to osmotic relationships. The latter point was also proved directly in an experiment in which osmotically equivalent amounts of sodium chloride, potassium nitrate, or magnesium sulphate were added to a staled medium, with widely different effects on the amount of sporulation.

It has already been mentioned that dilution of the fresh medium increases sporulation, and it has been shown above that reduction of concentration of certain of the food substances in the original medium has a similar effect. Such a reduction in concentration of food substances must necessarily have been brought about in the staled media by the organism acting as staling agent. This in itself is insufficient to account for the stimulatory effects of staled media, since an increase in sporulation far greater than the maximum brought about by dilution of the fresh medium was observed in certain cases. Moreover increased concentration of the staled medium led to increased sporulation.

The results of the present paper and of a further one in course of preparation are readily interpreted on the hypothesis that many fungi produce a substance or substances which stimulate sporulation. In the culture solution in which a fungus has grown there would, therefore, be three sets of substances, viz. the stimulatory substances referred to, staling products which tend to slow down growth, and unused constituents of the original medium. It is known from the work described above that the last of these, and especially glucose, tend to negative the action of

the stimulatory factor. The effect of staling substances would be to depress the activity of the *Melanospora* if they were present in sufficient quantity, i.e. if the cultures from which the media were prepared were old or if the 'contaminant' fungus were of a strongly staling character. By the interplay of the three factors indicated the behaviour of the *Melanospora* in mixed culture and on staled media, as described above, can be explained. Thus, in mixed cultures, it was noted (p. 333) that there was a delicate balance between the *Melanospora* and the contaminant which was upset if either colony were allowed to develop at the expense of the other. It may be assumed that slow growing organisms are only able to influence the sporulation of *Melanospora* if given time to develop sufficiently to enable an effective quantity of the spore promoting substances to be formed. Strongly staling organisms, however, are liable to produce sufficient staling substances to inhibit perithecial formation if they have been growing for some time before contact with the *Melanospora* colony takes place.

The response of the fungus to certain staled media can also be explained by a consideration of the interplay of the three sets of substances mentioned above. The favourable effects of an increased concentration of the staled media can be attributed to an increased concentration of the stimulatory substances, while the poor effects of media prepared from old cultures (Table III) can be explained by the presence of staling substances. The increased stimulation afforded by the ether-insoluble fraction of a staled medium (Table VI) is then explained by the removal of these substances by the ether. Since the fractionation with ether was carried out in acid solution it is probable that organic acids would be the substances in question. The further consideration of the nature of the active principle will be given in a later paper.

The active principle, it will be noted, is produced by *Melanospora* itself (p. 336) but not so rapidly or in such large quantity as it is produced by certain other fungi. On this account, presumably, a beneficial effect on sporulation is shown in the presence of certain other organisms or of their products.

## 7. SUMMARY OF RESULTS.

1. The formation of perithecia by *M. destruens* is markedly influenced by the composition of the nutrient media used. Details of the effects of various constituents are given in the text.
2. Transference of the fungus from one medium to a weaker one stimulates perithecial formation.
3. Temperature, light, pH concentration of the medium, mechanical checks to growth have, within wide limits, little effect on sporulation.
4. Sporulation of *M. destruens* and some other Ascomycetes is stimulated by the presence in the culture plates of certain other organisms or of

products derived from the latter. The activity in this respect of various 'staled' solutions increases with the degree of concentration but is diminished by the addition of a sufficient amount of various food substances.

5. Particularly active stimulation of fruiting is brought about by the ether-insoluble fraction of 'staled' solutions.

6. It is concluded that the intensity of sporulation in a 'staled' medium is determined by a combination of three factors, viz. reduction in food concentration by the organism used as 'staling agent', the production by the organism of inhibitory substances, and the production of a substance or substances which stimulate perithecial formation and which are formed but slowly by *M. destruens* itself.

The writers wish to express their thanks to Professor W. Brown for suggesting this investigation and for his interest and direction during its progress.

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## EXPLANATION OF PLATE V.

Illustrating the paper by Dr. Asthana and Dr. Hawker on 'The Influence of Certain Fungi on the Sporulation of *Melanospora destruens* Shear and of Some Other Ascomycetes'.

Perithecia, where present, can be seen with the aid of a hand lens as black dots. In Figs. 4-9 the *Melanospora* inoculum is the lower one in each case.

The Petri dishes used were 10.5 cm. in diameter.

Fig. 1. *M. destruens* on Medium A, 10 days after inoculation. No perithecia formed.

Fig. 2. *M. destruens* on Medium A, 16 days after inoculation. Numerous perithecia formed.

Fig. 3. *M. destruens*, 10 days after inoculation, original medium in plate = Medium A, medium in segment = 0.1 strength of Medium A.

Fig. 4. *M. destruens* and *Penicillium* sp. on Medium A, 7 days after inoculation. Note ring of perithecia.

Fig. 5. *M. destruens* and *Monilia fructigena* on Medium A, 12 days after inoculation. Note ring of perithecia.

Fig. 6. *M. destruens* and *Sclerotinia trifoliorum* on Medium A, 12 days after inoculation. Note clear zone between colonies.

Fig. 7. *M. destruens* and *Gloeosporium fructigenum* on Medium A, 12 days after inoculation. Note clear zone between colonies.

Fig. 8. *M. destruens* and *Helminthosporium* sp. on Medium A, 12 days after inoculation. Note clear zone between colonies.

Fig. 9. *M. destruens* and *Sphaeropsis malorum* on Medium A, 12 days after inoculation.

Fig. 10. *M. destruens*, 10 days after inoculation. Original medium in plate = Medium A. Medium in segment = medium staled by *Penicillium* sp. A few perithecia can be seen along line of junction of the segment.

Fig. 11. *M. destruens*, 10 days after inoculation. Original medium in plate = Medium A. Medium in segment = ether-insoluble part of medium staled by *Penicillium* sp. Numerous perithecia can be seen along line of junction of the segment.

Fig. 12. *M. destruens*, 10 days after inoculation. Original medium in plate = Medium A. Medium in segment = ether-soluble part of medium staled by *Penicillium* sp. Perithecia can be seen at periphery of segment (cf. Fig. 3). The dark patch along the line of junction is here due to dark hyphae and not to perithecia as in Fig. 11.





# The Absorption and Accumulation of Solutes by Living Plant Cells.

## VIII. The Effect of Oxygen upon Respiration and Salt Accumulation.

BY

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With five Figures in the Text.

RECENT approaches (see 3, 5, 10, 11, 16, 17, 20-27), both from the theoretical and experimental side, to the problem of salt accumulation reveal an increasing recognition that any satisfactory explanation must take adequate account of the role of metabolism. As yet, however, there has not emerged from the divergent views of different investigators any explanation which covers all the facts and commands general acceptance. Until this develops simultaneous comparisons between these apparently diverse aspects of physiological behaviour provide the only safe basis for theory. The data in this paper extend earlier observations upon the effect of oxygen concentration on the respiration and salt absorption of potato discs (23) to other tissues, and thus permit broader generalization concerning this important approach to the principal problem.

The results obtained with thin discs of potato tissue in aerated solutions demonstrated that both their respiration and salt absorption were conditioned by external oxygen concentration. The evident similarity between these two responses to oxygen concentration was one of several facts which prompted the view that salt accumulation by these living cells involved their metabolic activities. Although the earlier results clearly revealed a general, but quite definite, similarity between the two responses in question, they did not indicate that a fixed, simple, quantitative relation between

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*total* respiration and salt absorption could be derived. Since then (2, 24) cases have been found in which high carbon-dioxide production was not accompanied by high salt accumulation. In the light of this and other evidence the conclusion that respiration consists merely in the production of carbon dioxide ( $H^+$ ; and  $HCO_3^-$ ) cannot be entirely justified, and in the view of the writers (2, 24) has been superseded by the conception that salt accumulation is essentially a property of cells possessing certain other attributes in addition to high respiration. In storage tissues these additional metabolic properties are best indicated, at present, by the fact that for rapid accumulation cells must also retain the ability to grow and divide. Granted the ability to grow, the capacity for accumulation follows. Though the absolute amounts of salt absorbed may often be increased by a salt deficit in the initial tissues (24), this is probably not important in the case of storage tissues except when the technique produces an excessive loss of solutes prior to absorption.<sup>1</sup> The principal factor in the salt absorption of storage tissues is therefore their ability for renewed active metabolism and even growth, and consequently the amount of salt accumulated is closely related to the rate of aerobic respiration, which represents the major source of energy for the whole system. The evident connexion between active salt accumulation and extensive oxidation of carbohydrate now seems to be beyond question<sup>2</sup> (13, 24), though the biochemical mechanism which connects these different phenomena is as yet unknown. These further comparisons between the effect of oxygen upon respiration and salt accumulation are then based, not on the view that carbon-dioxide production *per se* is the essential link between metabolism and salt absorption, but rather on the view that it represents the most convenient measure of the metabolic activity of the system.

In certain cases (18) the effect of oxygen upon the respiration of plant tissues is complex, and the study of this variable is a prominent feature of recent attempts to elucidate the mechanism of respiration. Despite the special conditions (e.g. tissue immersed in an aqueous medium) imposed by the necessity for obtaining simultaneously data of both respiration and salt absorption, the data here presented are relevant also to the more general problem of the effect of oxygen concentration upon respiration. For thin discs of potato there is an evident region of proportionality between oxygen pressure and respiration. In the higher pressures, above a critical oxygen concentration, respiration becomes virtually independent of oxygen. Similar curves obtained by Chevillard *et. al.* have been analysed mathematically by Pei-Sung Tang (28). Other experiments upon the excised roots of

<sup>1</sup> Unpublished experiments show that the salt subsequently accumulated by potato discs may be increased by protracted washing in running tap water.

<sup>2</sup> This conclusion is not only derived from the published results, but receives confirmation by much unpublished evidence.

barley grown in water culture (13), and also on the shoots of *Elodea* (19) have substantially confirmed the conclusions derived from potato. An interesting difference is, however, apparent. These tissues only become limited in their respiration and salt absorption at concentrations considerably lower than is the case for potato discs. In this paper results are given for two other storage organs, a root (carrot) and a stem (artichoke), which thus offer an interesting comparison with the earlier results—a comparison which gains added force when it also includes the results here reported for the roots of potato grown in water culture.

The methods adopted for the experiments with storage tissue have been described elsewhere (21).<sup>1</sup> In this work only discs of one thickness (0.085 cm.) were used. However, this must not imply that the striking effects associated with the thickness and surface of discs, so prominent a feature of the potato experiments, are not shown by these tissues. On the contrary, data available, but not here presented, indicate that they are equally important. This fact must be borne in mind in the interpretation of the results.

Both the storage organs chosen, namely, carrot (*Daucus carota* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.) will accumulate the bromide ion from dilute aerated solutions of potassium bromide, and both have cells which retain the capacity for renewed growth and cell division to which attention has been directed elsewhere (2). The former tissue is of particular interest because of the recent work of Briggs and Petrie (4) and its frequent use by other investigators, without, however, the special attention to aeration which is essential; the latter because of its pronounced time drift in respiration and salt absorption (26). As these and other data show, carrot is not free from this time drift, but the effect of oxygen upon it will be discussed mainly with respect to the more pronounced example in artichoke. Briggs and Petrie (4) make some reference to the effect of oxygen concentration on both respiration and salt accumulation by carrot tissue, but the data leave much to be desired with regard to both problems. If the effect on carrot tissue resembles that found for potato then the choice of gas mixtures (air, oxygen, nitrogen) could not adequately reveal the oxygen effect. Furthermore, the use of the same batch of tissue exposed to different oxygen tensions at arbitrarily chosen points along a time drift is not as suitable as experiments with parallel controlled cultures which differ throughout only in one particular—namely, oxygen concentration.

#### EXPERIMENTAL DATA.

*The effect of oxygen upon respiration.* The essential feature of the data previously presented for potato respiration was the steep, approximately

<sup>1</sup> All the methods of analysis were as described earlier, except that potassium was determined by the procedure of Hibbard and Stout (9).

linear increase of respiration from the low value obtained in solutions in equilibrium with 2.5 per cent. oxygen up to a high and maximum value attained when the aerating gas stream had the composition of air. Further increase of oxygen pressure produced but little effect upon the carbon-dioxide production. An increased respiration associated with decreased

TABLE I.

*Effect of Oxygen Tension and Time upon Respiration of Carrot.*

40 discs (mean thickness 0.086 cm.) in 2 litres of 0.00075 N KBr at 23° C.

Oxygen %.	Initial weight gm.	Period.	Hours.	CO <sub>2</sub> (mg. per gm. hr.).
2.65	28.47	1	16.7	0.163
		2	24.0	0.126
		3	24.0	0.094
		4	23.9	0.095
		1 to 4		0.116
12.2	28.63	1	17.0	0.241
		2	23.8	0.289
		3	23.8	0.208
		4	23.8	0.165
		1 to 4		0.208
20.8	27.95	1	17.0	0.277
		2	23.7	0.310
		3	23.7	0.274
		4	23.7	0.208
		1 to 4		0.267
43.4	28.20	1	17.1	0.252
		2	23.7	0.377
		3	23.7	0.296
		4	23.8	0.201
		1 to 4		0.284

oxygen concentration, attaining a high value with nitrogen, which might have been anticipated from the results obtained by other workers (usually with massive tissues respiring in a gaseous environment (7, 8, 18) was never observed when the tissue was kept healthy and free from infection. The effects of oxygen on respiration and bromide accumulation were very similar, so that when both processes were calculated upon a relative basis (value for the culture in equilibrium with air = 100) the two curves were nearly superimposable. The deviation between them is explicable if salt absorption is related solely to aerobic respiration, and if an increasing proportion of anaerobic respiration contributed to the carbon-dioxide production at low oxygen pressures. With due allowance for certain complicating factors it is also clear that both anion and cation absorption were affected by the oxygen treatment.

*Carrot.* The results obtained with carrot tissue are given in Table I and Fig. 1.

It is evident that the general form of the oxygen-respiration curve is the same as that found for potato (see mean rate for periods 1 and 4, Fig. 1),

and, again, that although respiration is greatly affected by oxygen at the lower partial pressures, it is but little affected by changes of oxygen concentration in the gas phase at values greater than 20 per cent. There is clearly no indication of increased carbon-dioxide output caused by reduced

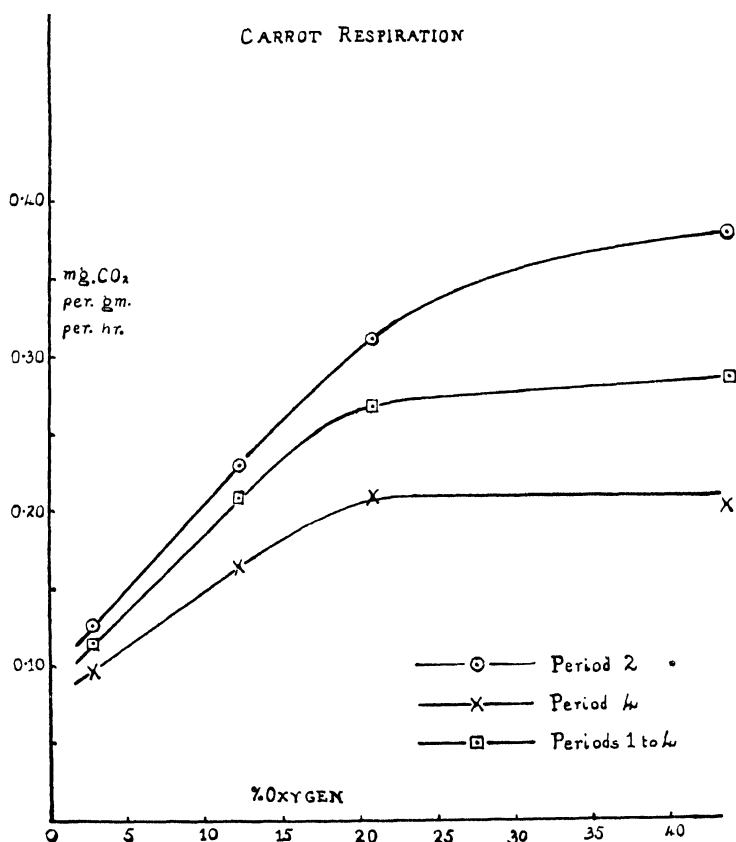


FIG. 1.

oxygen concentrations. Even should this appear at still lower oxygen pressures this result would have no theoretical significance if it were due to anaerobic organisms present as contaminations (see 23). The absence of any pronounced effect on respiration consequent upon a change from air to pure oxygen in the experiments of Briggs and Petrie is clearly explicable. However, there is in our experiments no parallel for Briggs's and Petrie's observation that after eleven days a change from pure oxygen to nitrogen caused a slight and progressive increase of respiration. The duration of the oxygen response here described (140 hours from the actual cutting of the carrot tissue) and the even longer interval which elapsed in the case of artichoke (12 days, see Table III) is evidence that these oxygen effects

persist and are not merely transient ones confined to a limited portion of the time drift. If increased respiration in low oxygen concentrations is ever a real feature of the respiration of storage tissues (carrot and artichoke—see Figs. 1 and 2, and compare potato) then it can only operate for so brief a period that it does not enter into these comparisons between respiration and salt accumulation.

TABLE II.

*Effect of Oxygen Tension and Time upon Respiration of Artichoke.*

40 discs (mean thickness 0.084 cm.) in 2 litres of 0.00075 N KBr at 23° C.

Oxygen %.	Initial weight gm.	Period.	Hours.	CO <sub>2</sub> (mg. per gm. hr.).
2.65	29.80	1	16.7	0.177
		2	24.0	0.084
		3	24.0	0.062
		4	23.9	0.048
		1 to 4		0.086
12.2	30.20	1	17.0	0.220
		2	23.8	0.138
		3	23.8	0.080
		4	23.8	0.061
		1 to 4		0.117
20.8	29.93	1	17.0	0.285
		2	23.6	0.157
		3	23.7	0.085
		4	23.6	0.063
		1 to 4		0.137
43.4	30.63	1	17.0	0.293
		2	23.7	0.191
		3	23.6	0.104
		4	23.8	0.069
		1 to 4		0.154

*Artichoke.*

In Table II and Fig. 2 respiration data are given which were obtained under conditions similar to those for the carrot experiment. It is quite evident that during the early periods when the highest respiration rates were recorded that the respiration was greatly affected by oxygen and in a manner almost identical with that previously described for potato (23) and carrot: respiration increasing with oxygen tension from the lowest concentrations utilized (2.65 per cent.) to values approximating to that of air. The total respiration decreases at all oxygen tensions with time, and in the later periods there is the suggestion that the effect of oxygen upon respiration extends to concentrations higher than that of solutions in equilibrium with air. This appearance is clearly due to a complex interaction of the time factor and the effect of oxygen which cannot be analysed completely on the basis of these data. The ultimate nature of the internal factor causally responsible for the behaviour with time is still obscure although it is, no doubt, associated with the maintenance of the supply of

the immediate respiratory substrate in artichoke. Unpublished experiments have shown that, although the respiration may be increased greatly by supplying sugar externally, the time drift cannot be obliterated entirely.

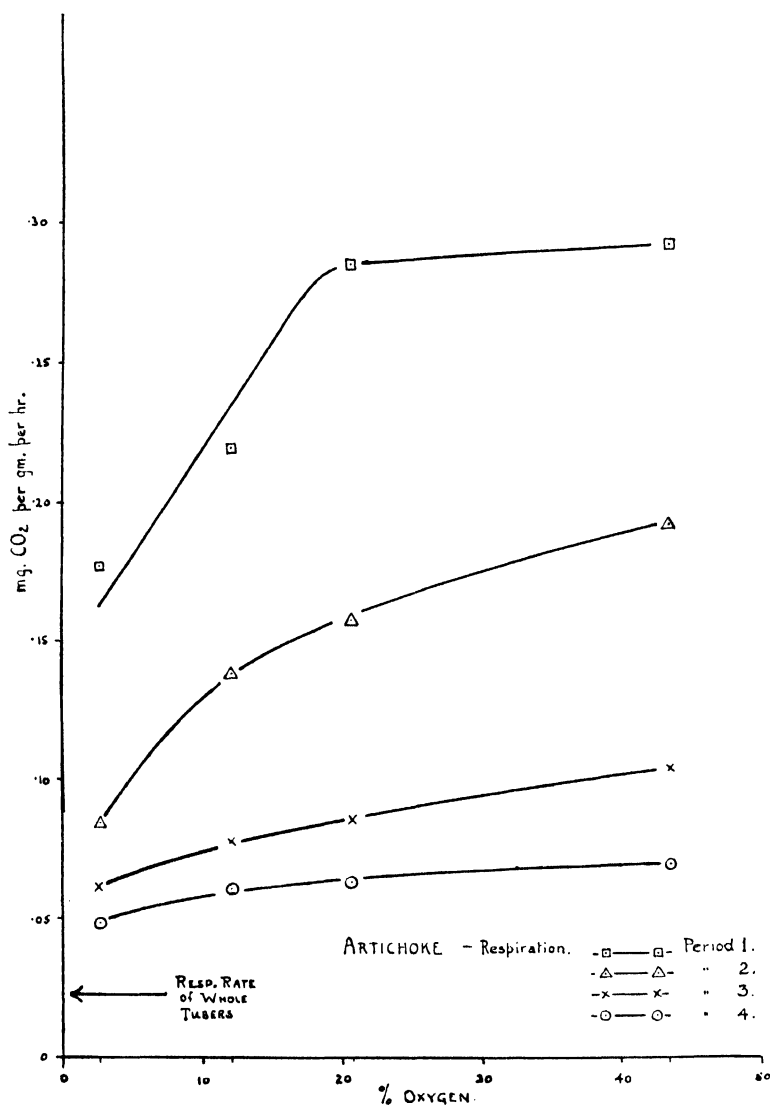


FIG. 2.

Clearly then the effect of oxygen *per se* can only be ascertained when the limitations due to the unknown factor are at a minimum i.e. in the first period. It is under these circumstances that the case of artichoke is similar to those of the other storage tissues examined.



The effect of oxygen on the time drift of respiration in artichoke suggests that after a protracted period, which may be estimated<sup>1</sup> at approximately 140 hours (for 23° and well aerated solutions), the respiration of artichoke discs should become almost independent of oxygen concentration. There is also a presumption that when this is attained the cut discs would have a respiration rate very similar, if not identical, with the whole tubers. Since this would imply that the surface cells of the artichoke

TABLE III.

*Effect of Oxygen Tension on Respiration of Artichoke After 265 Hours in Aerated Solutions.*

40 discs (mean thickness 0.078 cm.) in 2 litres of 0.00075 NKBr at 23° C.

Oxygen %.	Hours.	CO <sub>2</sub> (mg. per gm. hr.).	[Br] in sap equivs. $\times 10^{-3}$ .	Rel. Br absorption.
2.25	48	0.052	5.35	22.6
20.8	48	0.068	23.7	100
50.0	48	0.078	25.6	108

discs were no longer exhibiting those properties of enhanced vital activity and metabolism with which salt accumulation seems to be inevitably associated in storage tissue (23, 24, 25) an attempt was made to obtain tissue in this condition. Unfortunately it was not practicable to maintain exactly the usual experimental conditions for the whole of this period, so the tissue was placed at room temperature in a shallow trough with unstirred unchanged solution and continuous but somewhat less efficient aeration. As the data show (Table III), even after 265 hours of this treatment the tissue, when removed to the usual experimental conditions still revealed during the ensuing 48 hours some response to oxygen, but its magnitude was less than that obtained in the first periods of the former experiment.

The effect of oxygen upon the respiration of artichoke discs may be summarized as follows:

The cut discs, in common with other storage tissues, examined by these methods, respire at a very much greater rate than the tissue present in the uncut tuber. Access of free oxygen to the surface cells is one of the most important factors in this initial high respiration rate. When internal factors permit a high rate the effect of oxygen is almost identical with that observed in the case of carrot and potato. Low respiration is then associated with the use of a gas of low oxygen concentration and a rapid increase of carbon-dioxide production accompanies an increase in the oxygen content of the solution, until, with solutions in equilibrium with air, other factors predominate and the tissue fails to respond to further increase of oxygen concentration. Progressively with time the carbon-dioxide production is increasingly limited by other factors than oxygen concentration and there

<sup>1</sup> By plotting the data of Table II as respiration-time curves and extrapolating.

is a strong presumption that eventually it would become almost independent of it when the respiration rate has declined approximately to that of the uncut tuber.<sup>1</sup> Despite the wide range of oxygen concentration examined, and the fact that the aqueous media accentuate the effects of low oxygen,<sup>2</sup> an increased carbon-dioxide production associated with a reduced oxygen tension has not been observed.

TABLE IV.

*Effect of Oxygen Tension upon Bromide Absorption by Carrot and Artichoke from 0.00075 N KBr at 23° C.*

Oxygen. %.	Carrot.		Artichoke.	
	[Br] in sap equivs. $\times 10^{-3}$ .	Rel. Br absorption.	[Br] in sap equivs. $\times 10^{-3}$ .	Rel. Br absorption.
2.65	9.2	42.3	6.3	17.3
12.2	18.6	85.5	27.6	76.2
20.8	21.8	100	36.4	100
43.4	25.7	118	37.6	103

TABLE V.

*Effect of Oxygen Tension upon Potassium Absorption by Carrot and Artichoke from 0.00075 N KBr at 23° C.*

Oxygen %.	Carrot.		Artichoke.	
	K absorbed from ext. soln. equivs. $\times 10^{-3}$ .	Rel. K absorption.	K absorbed from ext. soln. equivs. $\times 10^{-3}$ .	Rel. K absorption.
2.65	0.01	22	0.07	12
12.2	0.44	96	0.43	74
20.8	0.46	100	0.58	100
43.4	0.54	117	0.58	100

*The Effect of Oxygen upon Salt Accumulation.*

*Bromide.* The experiments, already described, which show the effects of oxygen upon the respiration of artichoke and carrot discs were made with tissue immersed in dilute (0.00075 N) potassium bromide solution. In the case of artichoke (27) and potato (23) it has been shown that the direct effect of this concentration of potassium bromide upon respiration is small and, for present purposes, negligible. The same may be inferred for carrot. The initial and final composition of the expressed sap or external solution permits the effect of oxygen concentration upon salt accumulation to be determined, and this may then be compared with the effects upon respiration. It is convenient to deal with the two tissues concurrently.

Table IV and Figs. 3 and 4 show that oxygen concentration influenced not only respiration but also the bromide accumulated in the sap from the

<sup>1</sup> Based upon actual measurements of respiration of whole tubers. Data not given here.

<sup>2</sup> 20 per cent. oxygen in the gas phase corresponds approximately to 0.6 per cent. dissolved oxygen.

dilute external solution.<sup>1</sup> Qualitatively the response is the same in both cases—the lower oxygen concentrations produce smaller carbon-dioxide output and simultaneously less bromide is accumulated in the sap. These results, therefore, confirm those previously obtained with potato and only

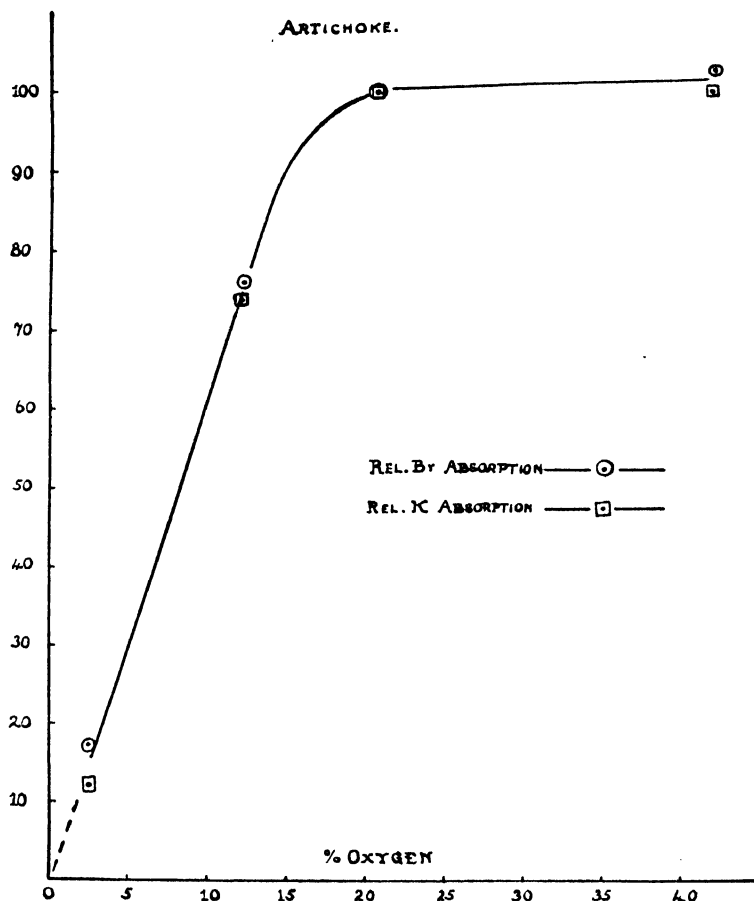


FIG. 3.

very minor differences in the case of carrot are to be noted (the critical limiting oxygen concentration is perhaps somewhat less defined and there is greater bromide accumulation at the very low oxygen tensions). Direct comparison between the respiration and salt accumulation curves is complicated by the time drift (especially in the case of artichoke) and to some extent by the surface effects to which earlier reference was made. Particularly in the case of artichoke the effect of oxygen upon bromide absorption is almost identical with that of potato (23, p. 212). Despite the rapid flow of

<sup>1</sup> Total halide as well as bromide was determined. These figures merely reflect the bromide figures and show no signs of chloride bromide exchange. The halide data are therefore omitted.

gas, oxygen concentration limits bromide absorption at all concentrations lower than that of solutions maintained in equilibrium with air. Comparison of Figs. 2 and 3 and the summary on p. 352 will recall that the effects of oxygen upon respiration and bromide absorption are very similar,

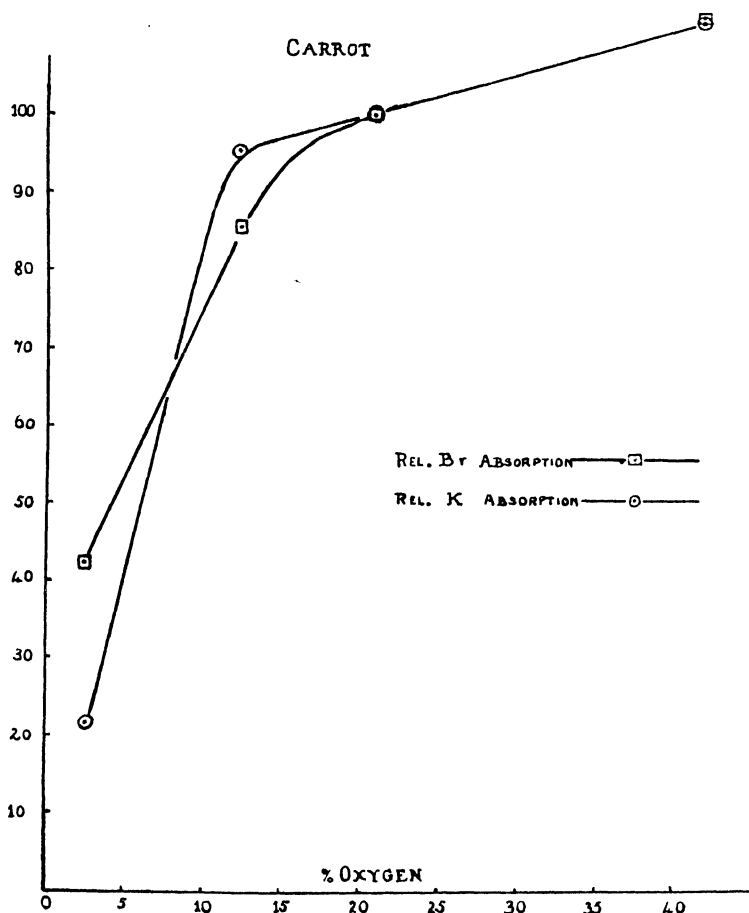


FIG. 4.

especially in the early periods before other factors [which also retard bromide accumulation (27)] intervene and limit the respiratory response to oxygen. When the respiration of artichoke discs has become so limited in its response to oxygen pressure by the operation of the 'time factor', then it appears (see Tables III and IV) that this also involves a reduced effect of oxygen upon bromide accumulation. It is, however, evident that the effect of oxygen upon bromide accumulation is relatively greater than upon *total* carbon-dioxide output. At low oxygen tension the former approaches zero,<sup>1</sup>

<sup>1</sup> Zero in the case of artichoke and a low value for carrot.

whilst the latter would still retain a considerable magnitude. A similar effect was previously observed for potato (23, p. 220). As for potato (23, pp. 235-40), so for artichoke, and no doubt for carrot, the inference is that bromide accumulation is essentially associated with aerobic metabolic processes. In both these cases there is again evident rather a suggestive parallelism between carbon-dioxide production and bromide absorption than a simple, direct relation between the two processes. Respiration and salt accumulation respond concomitantly to changes in oxygen tension because both are dependent upon oxygen and the metabolic processes which it determines. It is clearly the latter rather than the effects of mere carbon-dioxide production which is causally concerned with accumulation (24).

#### *Potassium.*

There remains, however, the effect of oxygen upon cation absorption. The previous data referring to potato (23, pp. 213-15) showed that cation absorption was also a function of oxygen pressure. The problem relative to potassium, however, is complicated by several factors which are not involved in the case of bromide. Of these the preliminary loss and subsequent reabsorption of potassium as well as the analytical difficulty involved in the determination of small changes in sap concentration in the presence of a high initial potassium content deserve special mention. When these difficulties were successfully overcome in the case of potato it became evident (23, see p. 215) that the effect of oxygen upon cation absorption was almost identical with that shown for bromide, with the addition that at low oxygen concentrations the tissue also lost potassium. It is clear that in lesser degree these complications also apply to the case of artichoke (27), and no doubt also to carrot. The complete evaluation of all the factors at different oxygen tensions for the two tissues now under discussion is a matter of some complexity, but it will suffice for present purposes to demonstrate that the effect of oxygen upon the behaviour of potassium is similar in kind to that already described for bromide. This became obvious when the total potassium absorption was obtained by difference from the initial and final potassium content of the external solutions. For this and other reasons it is not surprising that on this basis the potassium absorption determined was less than equivalent to the corresponding bromide absorption. However, equal or unequal absorption of anion and cation is not here a primary concern but, since Lundegårdh, who postulates a direct connexion between respiration and anion absorption (14, 15), denies that any such relation exists for the cations, it is of considerable interest that in these cases, as in the earlier experiments upon potato, greater potassium absorption is clearly associated with the higher oxygen tensions which simultaneously produce maximum aerobic respiration and maximum bromide accumulation. In Table 5 the potassium absorption under the influence of the different oxygen

tensions is given both in absolute units and also upon a relative basis so that the tissues may be compared directly. For the relative basis the absorption which occurred in solutions in equilibrium with air is arbitrarily designated 100. Figs. 3 and 4 show clearly that the effect of oxygen upon potassium absorption is for both tissues similar to that upon bromide absorption and again strongly resembles the effect previously described for potato. Apparently at all oxygen concentrations below that in solutions in equilibrium with air, oxygen limits the cation absorption. It is noteworthy that both these tissues seem able to survive oxygen deficit with less evidence of potassium loss than the potato tissue, which, as previously emphasized (25) is strongly aerobic. In both cases potassium absorption rapidly approaches zero at zero oxygen concentration.

*Effects of Oxygen Pressure on Respiration and Salt Absorption by Roots.*

Thus far the results have so confirmed the essential features of those obtained with potato discs, both with respect to the form of the oxygen respiration, and oxygen-salt absorption curves and the approximate value of the limiting oxygen concentrations that an extended generalization appeared to be in sight. However, similar experiments, then unpublished, by Hoagland and Broyer whilst they provided a general confirmation, directed attention to one interesting difference. Excised roots from barley grown in water were obtained under conditions of light and salt supply such that a tissue very active in salt absorption was obtained. It was found that the effect of oxygen concentration upon this system relative to potassium, nitrate, and halide absorption as well as respiration and sugar metabolism, was identical in kind with that already described, but oxygen operated as a limiting factor only at concentrations much lower than for the cases of potato, carrot, and artichoke (of the order of water in equilibrium with 5 to 10 per cent. oxygen rather than air). This might be dismissed as a specific property of barley, but a similar observation was made by Rosenfels (19) for the case of the shoots of *Elodea*. Since the storage tissues examined included both modified stems and roots, purely morphological explanations are hardly adequate. The essential difference appears to be rather that all the storage tissues examined develop virtually in an aerial environment, whereas those tissues which were shown to withstand successfully lower oxygen concentrations without limitations upon their respiration and salt absorption, had developed in an aqueous medium. In short, this appeared to be a problem not concerned with the difference between storage organ and growing root, or even with the specific properties of different plants, but rather a property dependent upon the conditions which obtained during growth. For this additional reason, and also because the root is the most important absorbing organ for salts, particular interest attaches to experiments upon the effect of oxygen tension upon respiration and absorption

by the roots of potato. These may then be compared directly with the known effects upon tissue from tubers.

For these and other experiments a large stock of uniform potato roots was obtained. About 100 lb. of uniform potato tubers were cut into seed

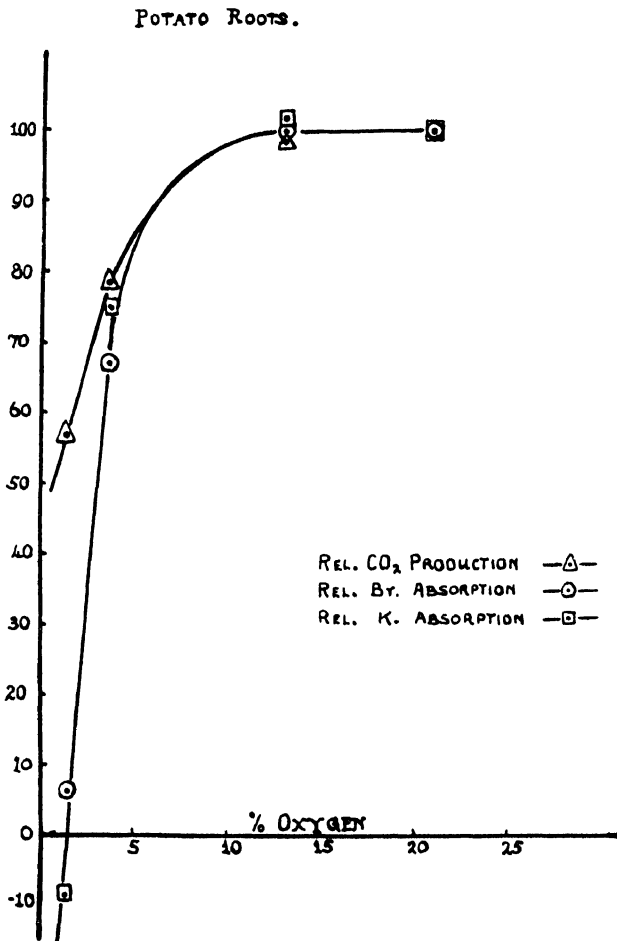


FIG. 5.

pieces, planted in sand, irrigated with nutrient solution and grown in the greenhouse until roots and shoots were established. They were then transferred to a large tank, equipped with aeration devices and containing a large volume (340 litres) of half-strength Hoagland's complete nutrient solution (12). The seed pieces were completely removed at this juncture. The small plants (about 4 to 6 in. apart) were supported on wire trays,<sup>1</sup> having layers of non-absorbent cotton to exclude light from the roots. Allowing

<sup>1</sup> The tanks and trays, &c., were standard fittings in the Division of Plant Nutrition of the University of California.

a period of adjustment to the new environment the nutrient solution was much diluted (approximately six times) when the roots were clearly in active growth. This procedure simulated the condition of low salt supply which Hoagland and Boyer find a desirable preliminary to salt absorption experiments with roots (13). Under this treatment the absorbing capacity of the roots for bromide increased progressively with time until eventually

TABLE VI.

*Effect of Oxygen Tension upon Respiration and Salt Absorption by Potato Roots.*

Tissue in 3 litres of 0.005 N KBr at 23° C.

Oxygen %	Initial weight gm.	Hr.	CO <sub>2</sub> (mg. per gm. hr.)	Rel. resp. rate.	Sap Final [K] equivs. ( $\times 10^{-3}$ ) per litre.	Rel. K absorp- tion.	Sap Final [Br] equivs. ( $\times 10^{-3}$ ) per litre.	Rel. Br absorp- tion.
1.40	37.9	24	0.093	56.7	17.6	-9.26	1.2	6.14
3.80	38.2	24	0.130	79.3	40.0	70.5	13.0	66.4
13.0	38.0	24	0.161	98.3	48.8	102	19.7	100
20.8 orig. tissue	38.0	24	0.164	100	48.3	100	19.6	100

20.2

\* Corrected for change in HCO<sub>3</sub> content of external solution.

extreme nutrient deficiency introduced irreversible injury. The first experiments (details not given) were made when the young roots were still rich in salts derived from the tubers and before the photosynthetic activity of the leaves was well established. The minimal salt absorption then obtained and the absence of any conspicuous effect of the oxygen concentrations then used upon respiration merely indicated that these limitations should be avoided<sup>1</sup> and a subsequent experiment (twenty-seven days later) which accomplished this is described here in some detail.<sup>2</sup> It is also necessary to recall that the absorption by excised roots may continue for a period unabated but is eventually followed by a progressive decline of activity which may even result in extensive loss of solutes. Experiment showed the duration of the period of maintained activity for the case of potato roots, and unless otherwise stated, this was not exceeded.

The experimental conditions resembled those of the storage tissue experiments. The root material was treated by methods used by Hoagland and Broyer for barley roots (13). The mixed roots from 150 plants (about 400 gm.) were random sampled into batches of approximately 38 gm. Free water was removed by centrifuging at slow speed for a standard time and the fresh weights could then be duplicated. As in the

<sup>1</sup> See general discussion of this procedure, Steward (24, pp. 528-31).

<sup>2</sup> The foliage was now showing definite, though not extreme, symptoms of nitrogen deficiency. Stolons had formed as well as numerous small tubers. Root systems, from which stolons and young tubers were removed, were large and much branched into numerous very fine laterals.



storage-tissue experiments the initial fresh weight was utilized as the basis for calculating the respiration rates and the salt absorbed during the experiment was determined by difference from the composition of the expressed sap from the initial and final tissue.

The data are given in Table VI, but to facilitate comparisons the effect of oxygen upon respiration, potassium and bromide absorption is shown (Fig. 5) utilizing a relative basis by arbitrarily designating the value of each of the three properties as 100 for the cultures in equilibrium with air.

The form of the oxygen-respiration curve is clearly that which has been obtained with all the other tissues examined by these methods. Again increased respiration with reduced oxygen tension is not observed even at low concentrations. At zero oxygen the tissue would still apparently produce a definite carbon-dioxide output, but unless the extrapolation of Fig. 6 from 3.80 per cent. oxygen to zero is unjustified, high respiration in nitrogen can be entirely excluded. The interesting point is that the respiration of potato roots is not limited by oxygen except at concentrations (solutions aerated with 5 to 10 per cent. oxygen) much lower than is the case for discs cut from tubers (solution aerated with air). The general level of respiration is of some interest. Under similar external conditions and on the weight basis, the roots respired at approximately the same rate as the tuber discs commonly used (0.75 mm. thick) though their specific surface would be much greater.

As Fig. 5 shows, in solutions in equilibrium with an atmosphere containing less than 10 per cent. oxygen, both bromide and potassium absorption are limited by oxygen. Again the type of curve is identical with that found for the various storage tissues, and it is clear that the relative effect is the same for anion and cation (see also Figs. 3 and 4). The important difference between these data and those for the storage tissues is that the limiting oxygen concentration is lower.<sup>1</sup> As previously found for potato tubers, the concentration of oxygen at which the tissue exhibits oxygen want in respiration is also that which induces limitations upon salt absorption, and these limitations become progressively more apparent as oxygen concentration is further reduced. The deviation between the curves for carbon-dioxide output and for salt accumulation are similar to those observed previously for potato discs and they again suggest that it is solely the aerobic phases of respiration and metabolism which are concerned with salt accumulation.

It will be evident from Table VI, though not from Fig. 5, that in these experiments the total absorption of cation (K) exceeded that of the anion

<sup>1</sup> As an indication of a possible relation between mineral nutrient supply and the response to oxygen it is of interest that earlier experiments with potato roots not subjected to the nutritional deficiency failed to show appreciable oxygen want except at concentrations even lower than the above.

(Br). Since this is clearly a property determined rather by the initial state of the tissue than the external oxygen supply it will not be discussed here at length.

#### GENERAL DISCUSSION.

The results relative to the effect of oxygen upon respiration require little further discussion.

Of particular interest with reference to current theories is the entire absence of higher respiration in lower oxygen concentrations. It is evident that all the tissues used contained cells which were either still growing (roots) or remained capable of further growth and active metabolism, though limited somewhat in this respect by oxygen (surface cells of discs of carrot, artichoke, potato). There seems, therefore, to be grounds for the suggestion that in so far as the effects of oxygen become the basis of general theories of respiration they should be applicable to still active cells under conditions compatible with growth and active metabolism,<sup>1</sup> as well as to massive, mature, senescent organs in which the cells have irreversibly lost this capacity (e.g. apple, tomato, &c.). The essential similarity between the effects of oxygen upon respiration here described and those previously published for potato indicates that this is a general and not an isolated result.

The lower oxygen concentrations tolerated without limitation upon their physiological properties by those tissues which develop directly in water (e.g. roots) has wider implications even than its direct bearing upon the physiology of roots, for it suggests that metabolic factors are involved in the adaptation to an aquatic or terrestrial environment.

Since these results confirm all those comments made in earlier papers (20-26) upon the general technique of absorption experiments especially with storage tissues, further discussion along these lines seems to be superfluous, despite the fact that Asprey (1) has again persisted with the worst features of the older methods and attempts to evade the implications of much of the recent work. The effects of oxygen concentration here described with other tissues again indicate that controlled aeration must be the first requisite in all experiments upon the metabolism and salt relations of plants, and especially so where storage tissues and roots are concerned.

Of most immediate interest are those aspects of these results which concern the relation between salt accumulation and metabolism. If there ever existed any reasonable doubt that the conclusions derived from the storage tissues were of general application this should be dispelled finally by the almost identical behaviour of roots.

The pronounced effects of oxygen upon the absorption both of anion and of cation, which are not merely similar in kind but apparently may

<sup>1</sup> Reference may be made to Leach and Dent [Proc. Roy. Soc. B, 116, 150-169] for data on the effect of nitrogen on the respiration of seedlings.

also be of the same relative magnitude [see especially Figs. 3 and 5, and compare Fig. 4 and data in (23)], disposes of any theory of salt absorption, such as that of Lundegårdh (14), which restricts the factor of metabolism only to the absorption of anions. Furthermore the parallelism between respiration and absorption here described, even as it concerns anions, must not be confused with the mechanism of 'Grundatmung' and 'Anionenatmung' of Lundegårdh. It is evident that we are here concerned not with the small (and at these concentrations negligible) effects due specifically to the salt, but with relatively large changes in a respiratory component determined primarily by variables other than the salt absorbed (e.g. oxygen concentration, temperature, sugar supply, &c.). These and other reasons prompted an earlier criticism of the general approach of Lundegårdh but the position is now further complicated by a recent analysis (15) of oxygen absorption, carbon-dioxide evolution, and anion absorption by seedlings which concludes that 'Anionenatmung', the respiratory component with which salt accumulation is alone associated, comprises both anaerobic and aerobic fractions (e.g. Aerobe k. A., Extra CO<sub>2</sub>) whereas 'Grundatmung' which has no direct bearing on salt accumulation is said to be wholly aerobic. At low oxygen concentrations the anaerobic fraction of the 'Anionenatmung' predominates (*loc. cit.*, see p. 229). Associated with this is the qualitative observation (*loc. cit.*, see p. 226) that 'die Grundatmung annähernd parallel mit der Oberfläche des Organs, während die Anionenatmung mit dem Volumen variiert'. This is a curious reversal of the position advocated in this and previous papers in which it is maintained that salt accumulation in storage tissues is closely linked to aerobic metabolic processes, and in particular to that component of the total respiration which is determined both by the available surface for oxygen penetration and the oxygen concentration in the surrounding medium. The results in this paper and those of Hoagland (13, 24) indicate that roots are not exceptions to this rule.

The results emphasize anew that it is the aerobic phases of metabolism which are closely concerned with salt accumulation. In this the new results are in agreement and are entirely confirmatory of those earlier ones obtained with potato discs. The entire absence of accumulation of anions and cations in the absence of free oxygen<sup>1</sup> [Figs. 3, 4, 5, and see (23), pp. 210-15] is adequate proof that aerobic reactions are essentially concerned. As implied earlier [(23) p. 222, and p. 226 and this paper page 348] this explains the observed deviations between the curves for carbon-dioxide output and salt accumulation. If the carbon dioxide due to aerobic breakdown of sugar were distinguished from that due to anaerobic processes a closer parallelism could no doubt be drawn.<sup>2</sup> However, this relation will

<sup>1</sup> With the exception of the slight absorption of bromide by carrot.

<sup>2</sup> If the anaerobic respiration is derived by extrapolating the curves to zero oxygen and if it is

not be pursued further because, as emphasized in earlier papers (24, 26, 27), it is clear that a consideration merely of carbon-dioxide production cannot supply the essential link between metabolism and accumulation. This point is rightly emphasized by Rosenfels (19).

An adequate realization of the above facts and their implications does much to discredit certain views which have recently received prominence. Any theory of salt accumulation which relates it directly to carbon-dioxide output fails to distinguish between aerobically and anaerobically produced carbon dioxide and treats them as equally effective. The data show that this is not so; and this, together with certain other difficulties previously referred to (24), as well as observations that high carbon-dioxide production does not always lead to rapid salt accumulation (2, 24), and the realization that green cells in the light may accumulate salts whilst they are importing rather than evolving carbon dioxide (24, 19), discredits simple explanations based upon the substitution of entering ions for issuing  $H^+$  and  $HCO_3^-$  (3, 5). The inability of cells to accumulate at low oxygen concentrations, despite a considerable anaerobic production of carbon dioxide, is not in harmony with the view of Osterhout (16, 17), who stresses the effect of carbon-dioxide production upon the reaction of the sap, and also its capacity to provide bicarbonate ions available for anion exchange. It was merely in these terms that Osterhout [(17), p. 984] regarded the earlier experiments upon potato, and harmonized them with his general theory. Clearly, however, with respect to both of the properties mentioned anaerobically produced carbon dioxide should be as effective as that of aerobic origin. Tissue limited by oxygen with respect to salt absorption may still produce carbon dioxide in quantities which ought to suffice on the mechanism suggested.

If, however, wholly or in part, the correlation between respiration and salt accumulation is related to the energy liberated in respiration then, per molecule of carbon dioxide, the anaerobic breakdown has only approximately one-tenth the value of the aerobic. Even so it is evident that the total energy release at zero oxygen may still be of the order of 4 to 5 per cent. of that in solutions in equilibrium with air. The results all agree that either at zero oxygen or a higher concentration, salt accumulation vanishes, and in fact actual loss of solutes previously absorbed may occur extensively. In our view these facts confirm previous statements in this series, and restrict further consideration of the role of metabolism in salt accumulation to the aerobic reactions. Presumably it is some phase of oxidative breakdown of sugar which is involved. One cannot yet determine whether it is

assumed that the anaerobic respiration rate is independent of oxygen concentration one can construct relative aerobic respiration curves analogous to those of Figs. 1 and 2 and Fig. 2 of (23). These are more nearly identical with the respective curves for salt accumulation but still deviate slightly at low oxygen pressures, principally no doubt because accumulation in both potato roots and discs ceases at a low but real oxygen concentration (1.0 to 2.0 per cent.  $O_2$ ), whereas the aerobic respiration only vanishes at zero oxygen.

solely the reactions which culminate in carbon dioxide, or other reactions, which also involve oxygen and respond similarly to oxygen pressure, but in which other products are formed from sugar, which are primarily concerned with salt accumulation. With the reservations set out in an earlier paper (23), it is still maintained that it is the energy value of the oxidation which is the factor most probably involved, but rather because this maintains the cell system in the active condition compatible with a high rate of metabolism and salt accumulation than merely because it supplies the energy expended in the accumulation alone. It remains to be seen whether some definite stage in the oxidative break-down of sugar may be specifically associated with salt accumulation, and thus segregated from the more general effects of oxygen which induce or maintain the necessary activity in growth. Unpublished data which properly concern another investigation suggest that this possibility may be realized.

#### SUMMARY.

1. The effect of oxygen concentration upon carbon-dioxide production and salt accumulation ( $K'$  and  $Br'$ ) has been examined in two storage tissues (carrot and artichoke), and roots of potato grown in water.

2. All the results are in essential agreement with those previously obtained for potato discs.

3. In all cases the respiration is only appreciably affected by changes in oxygen concentration in the rapidly flowing gas-stream when concentrations lower than that of air are involved. Cells which develop directly in water (potato roots) withstand, without limitation of either respiration or salt absorption, lower concentrations of oxygen than the discs of storage tissue.

4. In the range of concentrations in which oxygen limits respiration, decrease of oxygen pressure invariably caused marked decrease of respiration. Increased respiration, due to reduced oxygen pressure, has not been observed, although very low oxygen concentrations have been utilized. High respiration in nitrogen is not then a general property, and may be restricted to cells which have permanently ceased growth and active metabolism.

5. The effect of oxygen upon the time-drift of respiration of artichoke and carrot discs immersed in water is described. With the former the conspicuous response to oxygen virtually disappears after a protracted period, by which time the respiration rate has declined to the very low value typical of that of the uncut tuber.

6. The oxygen concentrations which limit respiration are also those which limit salt absorption. Below these both potassium and bromide accumulation are retarded and apparently in the same degree.

7. It is clear that in all cases (potato, artichoke, carrot discs, and potato roots) that aerobic only and not anaerobic metabolic processes are related

to salt accumulation. Potassium and bromide accumulation vanish either at zero oxygen (artichoke, carrot discs) or at a low but slightly greater oxygen concentration (potato discs, potato roots).

8. It is again emphasized that the direct effects of mere carbon-dioxide production are not alone involved. Aerobic metabolism is believed to be that of a potential source of energy, not merely for the salt accumulation process alone, but rather to maintain the vital activity and growth with which salt accumulation seems to be inevitably associated.

The storage tissue experiments were part of extensive investigations pursued in the Botany Dept. of the University of Leeds, and the root experiments were undertaken as part of similar studies in the Division of Plant Nutrition, University of California. For the facilities provided by both laboratories our thanks are due, and especially to Professors J. H. Priestley and D. R. Hoagland, who gave this work every possible encouragement and support.

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# Physiological Studies in Plant Nutrition.

## IV. Nitrogen Metabolism in Relation to Nutrient Deficiency and Age in Leaves of Barley.

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### INTRODUCTION.

TO understand more fully the results obtained in certain published experimental work on growth and respiration rate in relation to nutrition (cf. 11, 34) it was desirable to know something of the internal composition of the plants studied, and in particular of their leaves. The nutritive elements considered were nitrogen, phosphorus, and potassium, and knowledge was required of variations induced in the distribution of the main nitrogen fractions by deficiency of any one of these elements. Published work on these lines proved to be unsatisfactory for the purpose, since not only had widely different plants been used by investigators, but the conditions of growth were usually dissimilar to those employed in the experiments under consideration ; indeed, in the case of potassium deficiency at



least, these differences were of such a magnitude in their effect on the type of plant produced that it was clearly dangerous to assume that results obtained by any particular worker could be applied to the type of plant considered here. Moreover the published results of other workers were not always consistent among themselves. A re-investigation of the whole question has therefore been undertaken. The present communication gives the results obtained in that investigation in so far as they concern nitrogen metabolism in relation to manurial supply and age, aspects of physiology which are as yet not sufficiently understood.

#### EXPERIMENTAL PROCEDURE.

Barley, *var.* Plumage Archer, was grown in a similar manner to that of previous experiments (11, 34). After sterilization with formalin the seeds were sown nine in a pot in sand culture on 3rd May 1931, and after germination the plants were thinned to three, selecting on the basis of uniformity and spacing. In all approximately 340 pots were used. The nutrients were applied immediately after germination, the complete amounts in grams per pot being as follows:

$\text{NaNO}_3$  9.1,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  2.52,  $\text{K}_2\text{SO}_4$  1.85,  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  0.37  
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.25.

The pots were divided into four sets, unequal in numbers in view of expected variations in leaf size and nitrogen content. Only one of them, the 'fully manured', received the above amounts of salts. The three other sets received reduced amounts of nitrogen, phosphorus, and potassium respectively. In each type of deficiency only one-tenth of the standard amount of the salt concerned was applied, while in the cases of reduced nitrogen and phosphorus the loss of sodium was corrected by addition of the requisite amount of sodium sulphate. By this means the only variable ion besides those under investigation was  $\text{SO}_4$ , and this was always present in adequate amount.

Eight samples of leaves were taken throughout the life history for nitrogen fractionation, sampling dates coinciding as far as possible with the complete emergence of eight selected leaves on the main axis. Usually eleven leaves are produced, but variation to ten or twelve is frequent; the last leaf is always small, and in these experiments ten only have been considered. With the treatment adopted the times of emergence of corresponding leaves in the various manurial sets differed by only a few days; the potassium deficient set had almost the same rate of leaf production as the control set, but with nitrogen and phosphorus deficiency there was a slight lag in the time of emergence of later leaves. In practice therefore a certain amount of compromise was necessary in the selection of later sampling dates, so that the selected leaves of the control and potassium

deficient sets had sometimes been expanded two or three days before sampling, while in three cases at least (phosphorus-deficient Leaves 6 and 9 and nitrogen-deficient Leaf 6) the corresponding leaves from the other two treatments were taken one or two days before expansion was complete. Samples were taken at the times of emergence of the successive first ten leaves, with the exception of Leaves 5 and 7. Sampling dates are as follows: May 29th, June 5th, 9th, 15th, and 24th, July 6th, 14th, and 28th.

At each sample all the living leaves on the main axis were collected and kept separate (i.e. all 1st, 2nd, 3rd, &c., were grouped separately), sufficient pots being used to ensure that the total dry weight in each collection of leaves should be 1.5–2.0 gm. Owing to the number of groups involved it was impossible to analyse the fresh material directly, and some method of preservation was necessary. Each batch was weighed fresh and dried rapidly at 70° C., the leaves being placed for some hours individually on perforated zinc trays in an oven through which a rapid stream of air was drawn. While still hot the samples were placed in dry warm bottles and immediately sealed. The weight of the samples at the time of opening the bottles was taken as the dry weight. At the same time small parallel samples of 2–3 leaves were taken and dried at 100° C. in order to estimate water content. The data procured from these leaves together with nitrogen estimations from the large samples are presented in Table II.

*Analytical methods.* The dried samples were ground immediately before analysis in a Wiley micro-mill. Total nitrogen was estimated on the ground material using the micro-Kjeldahl apparatus of Parnas and Wagner as described by Pregl (31). The remainder of the sample, usually about 1.5 gm. was ground thoroughly in a mortar with phenol-water and the extract filtered through paper-pulp. Using small apparatus and the minimum of paper-pulp, and exercising economy in the use of washing water, it was found that after adequate grinding complete extraction could be obtained in a very small volume. In these circumstances with practice it is possible to extract 97–98 per cent. of the soluble, crystalloid nitrogen in 50 c.c. of water; this does not refer to the protein nitrogen in the extract.

Protein was removed from the solution by adding to it 5 per cent. of a 50 per cent. solution of trichloroacetic acid and filtering. Total crystalloid nitrogen was estimated on the filtrate by the same micro-Kjeldahl technique. In all Kjeldahl estimations nitrate was included by an adaptation to the micro-scale of the reduced iron method of Pucher, Leavenworth, and Vickery (32). Aliquots of the cleared extract were also taken for the estimation of amino, ammonia, amide, and nitrate nitrogen. Amino-nitrogen was determined by an adaptation of Brown's modification (1) of the Sørensen formol-titration method. Ammonia was estimated after neutralization with magnesia cream by Wolff's method (46), and amide-nitrogen in a similar manner after hydrolysis with sulphuric acid and neutralization

with soda, as recommended by Vickery and Pucher (42). Finally nitrate was determined on the alkaline residues from the amide estimations by the addition of Devarda's alloy, reduction being completed in the cold after 20 hours; the ammonia was estimated by Wolff's method as before. All estimations, with the exception of amino-nitrogen and ammonia, were duplicated.

The preservation of leaves for nitrogen partition by desiccation is of course not ideal, and indeed Nightingale (23), on the basis of results obtained by Chibnall (4), has condemned it as useless. But these particular experiments of Chibnall's were designed to obtain maximum effects of drying, and the conditions of desiccation were very different from those in use to-day: drying was carried out at 40–50° C. at such slow rates that 50–120 hours were required. Naturally hydrolysis resulting from such treatment is sufficiently great to invalidate any results obtained by this particular use of the method. But the desiccation method has been used with success by several workers, including Thomas (38) and Tottingham, Schulz, and Lepkovsky (40), and it was on Professor Chibnall's advice that it was adopted in the present experiments.

The effects of drying were investigated in seven experiments, which included all the manurial types involved. Samples of fresh leaves were extracted and analysed immediately after gathering, while duplicate samples were dried, sealed up, and analysed after a similar storage period had elapsed to that in the main experiment. Apparently two types of hydrolytic changes occurred: (1) a certain amount of amide gave rise to free ammonia, and (2) protein was hydrolysed yielding amino acids. As regards the first, the sum of ammonia and amide-nitrogen was apparently unaffected by drying. Since in all fresh leaf samples ammonia was found in traces only, it has been assumed that the ammonia in the dried samples was derived from amide in the living leaves, i.e. all ammonia found in the extract after hydrolysis has been assumed to come from amide, an assumption which can involve no great error. Professor Chibnall has pointed out to the authors that in samples of rapidly dying leaves the error may be larger since ammonia produced by deamination may not then be so rapidly converted into asparagine. But large accumulations of ammonia relative to amide cannot have occurred since the directly estimated ammonia in all dried samples throughout the experiment was of a much lower order than that of the ammonia estimated after hydrolysis. Arguments subsequently used involving considerations of amide concentration or of the ratio of amide- to amino-nitrogen are unaffected by such ammonia accumulation as is possible.

The hydrolysis of a certain amount of protein, yielding amino-nitrogen, is rather more serious. The relative change in protein content is negligible, being of the order of 1 per cent., but the much smaller amount of amino-

nitrogen is increased considerably, by approximately 15 per cent. With the possible exception of potassium deficiency there appears to be little consistent difference between the manurial groups in the increase due to drying, and it is justifiable to compare the widely divergent figures among themselves, though the absolute level of all is too high. Two lots of fresh leaves from potassium-deficient plants were compared with duplicate lots of dried leaves, and these two comparisons gave the smallest increases of amino-nitrogen due to drying observed throughout. If this indicates a real manurial effect conclusions drawn subsequently from a consideration of the ratio of amide- to amino-nitrogen in the various groups are in no way invalidated. Later in this paper some emphasis is laid on the fact that this ratio as determined from dried samples is not higher in newly expanded leaves under potassium deficiency than under conditions of complete nutrition; it follows therefore that if there is any differential manurial change due to drying, this conclusion must hold with even greater force in fresh material.

#### PRELIMINARY REMARKS.

Before presenting the nitrogen results a few remarks are necessary concerning the samples of leaves analysed. The general symptoms of deficiency were similar to those previously described (11). In many characteristics deviations from the fully manured type of plant found under phosphorus and nitrogen deficiency were similar; in particular may be mentioned a marked reduction in the number of tillers produced and in the size of individual leaves, together with a reduced rate of leaf production. Such differences reflect diminished meristematic activity, and in these respects the potassium-deficient plants of the present experiments were in marked contrast with the other two deficient types; tillering and leaf production rates, and leaf size were here approximately normal. The most striking external symptoms of potassium deficiency were a light green colour, reduction in stem length, very early death of leaves and increased succulence of the plant. There was a failure to produce ears, and rapid death of tillers occurred at the stage at which stem elongation normally would begin; but in contrast with the other manurial types a second cycle of tillering began and the plant remained in a juvenile state until death.

Under nitrogen deficiency light green leaves were also found, but associated with decreased succulence, while the individual leaves persisted nearly as long as those of fully manured plants. Under phosphorus deficiency a dark dull green was developed together with considerable anthocyanin production, particularly in older parts. Early death of leaves occurred, though not so early as under potassium deficiency. Water content was nearly normal. The external symptoms of phosphorus starvation developed with great rapidity at a particular stage in the life history, and it is later shown that this change was accompanied by an equally sudden

modification of the internal composition. Later in the life history some measure of recovery was found, the most severe external symptoms being apparent about the time of Samples 5 and 6. Leaves 5 and 6 were the most adversely affected of those on the main axis.

TABLE I.

*Remarks Concerning Leaves Analysed.**Sample 4.*

## Leaf 1.

- F.M. A few tips yellow.  
 -N Less yellow than in F.M.  
 -P More yellow than in F.M.; some reddening.  
 -K Less yellow than in F.M.

*Sample 5.*

## Leaf 3.

- F.M. Tips yellow.  
 -N Tips yellow.  
 -P Approx.  $\frac{1}{3}$  dead.  
 -K Approx.  $\frac{1}{3}$  dead.

## Leaf 4.

- —  
 Tips dead.  
 Tips dead.

## Leaf 6.

- Not fully expanded.  
 Not fully expanded.  
 —

*Sample 6.*

## Leaf 5.

- F.M. Tips yellow.  
 -N Approx.  $\frac{1}{3}$  dead.  
 -P Approx.  $\frac{3}{4}$  dead.  
 -K Approx.  $\frac{3}{4}$  dead.

## Leaf 6.

- —  
 Approx.  $\frac{1}{2}$  dead.  
 Tips dead.

## Leaf 7.

- —  
 —  
 A few tips yellow.

*Sample 7.*

## Leaf 6.

- F.M. Tips dead.  
 -N Approx.  $\frac{1}{3}$  dead.  
 -P Approx.  $\frac{2}{3}$  dead.  
 -K Wholly dead.<sup>1</sup>

## Leaf 7.

- —  
 Approx.  $\frac{1}{2}$  dead.  
 Approx.  $\frac{1}{2}$  dead; variable.

## Leaf 8.

- —  
 —  
 Approx.  $\frac{1}{3}$  dead.

## Leaf 9.

- —  
 Not fully expanded.  
 —

*Sample 8.*

## Leaf 7.

- F.M. Approx.  $\frac{1}{3}$  dead.  
 -N Approx.  $\frac{2}{3}$  dead; variable.  
 -P Wholly dead.<sup>1</sup>  
 -K Wholly dead.<sup>1</sup>

## Leaf 8.

- Tips yellow.  
 Approx.  $\frac{1}{3}$  dead.  
 Wholly dead.<sup>1</sup>

## Leaf 9.

- —  
 —  
 Approx.  $\frac{2}{3}$  dead.

In Table I are given a few observations concerning those samples of leaves which were either not completely expanded at the time of sampling or were dying. It should be remembered that death of leaves in barley starts at the tip and proceeds downwards in the same way as the original expansion. Hence ' $\frac{1}{3}$  dead' implies that the distal third of the leaf was shrivelled and dry. The fraction refers to leaf length.

It should be stated that at an early stage of growth some of the plants unfortunately developed chlorosis due to iron or possibly to manganese

<sup>1</sup> Not sampled.

deficiency. This was particularly noticeable in the fully manured series and occurred at the time the third leaf was expanding. This leaf, and to some extent the second also, developed lighter or yellow-green stripes, and in some instances a few small brownish spots appeared. An application of iron and manganese corrected the condition. Subsequent leaves were normal in appearance while affected leaves became a uniform dark green. This circumstance was probably not without an effect on nitrogen metabolism at least in the fully manured series, and aberrant figures in early leaves presumably due to this cause are pointed out later.

The results are presented without statistical evidence. Although lack of replication of observations, together with obviously high interactions between the factors concerned, renders rigid statistical treatment impossible, nevertheless a statistical examination of the results was made, and only such deductions as are supported by the analysis are stressed in the sequel. Since this evidence merely confirms conclusions which appear reasonable from an inspection of the figures, and in its presentation would occupy much space, it has been omitted. In order to analyse the results each nitrogen fraction was considered individually. Three variables are then concerned: manurial treatment, leaf number, and leaf age. The only possible estimate of error variance must include also variance due to the highest order interaction, and this appears to be often very large. In order to reduce the magnitude of this interaction as far as is reasonably possible, the first three leaves, in which manurial differences either do not exist or are very slight, were omitted, and a symmetrical table obtained by considering the first two samplings of every leaf from 4-9 inclusive. The total variance was then analysed into the following components:

	Degrees of freedom.	Interactions.	Degrees of freedom.
Treatment	3	Treatment $\times$ number	15
Leaf number	5	Treatment $\times$ age	3
Leaf age	1	Number $\times$ age	5
		Error	15

The estimate of error so obtained is a maximal one and therefore may presumably be safely applied to figures not included in the symmetrical table.

#### RESULTS.

The results are presented in Tables II and III, as percentage Dry Weight (Table II) and percentage Total Nitrogen (Table III).

(1) *Total nitrogen.* Total nitrogen figures give the integrated difference in amount of nitrogen supplied to, and leaving the leaf up to a certain time compared with the dry weight of the leaf at that time. Since the nitrogen was applied to the pots some time before the first sample, and is rapidly taken up by the young plant, while the increase in weight throughout life history tends continually to diminish the total internal concentration, the

TABLE II.

Leaf, sample, and treatment.	Water.	Total N.	Protein N.	Crystal- loid N.	Total amino N.	Amide N.	Amide as % amino N.	Nitrate N.	Residua crystal- loid N.
F.M.	619	4.92	3.95	0.971	0.263	0.059	22.4	0.387	0.262
L. 1 -N	602	4.81	3.90	0.914	0.263	0.046	17.3	0.248	0.357
S. 1 -P	629	4.91	3.84	1.07	0.291	0.053	18.3	0.419	0.303
-K	698	4.79	3.82	0.973	0.264	0.061	22.9	0.402	0.246
F.M.	548	4.70	3.74	0.957	0.342	0.104	30.4	0.339	0.172
L. 1 -N	539	4.65	3.69	0.956	0.334	0.069	20.7	0.240	0.313
S. 2 -P	491	4.05	3.10	0.952	0.345	0.087	25.2	0.264	0.256
-K	580	4.07	3.14	0.931	0.314	0.067	21.3	0.307	0.243
F.M.	628	4.72	3.82	0.899	0.265	0.063	23.8	0.287	0.284
L. 1 -N	579	4.36	3.58	0.779	0.319	0.057	17.8	0.156	0.247
S. 3 -P	642	4.31	3.47	0.837	0.322	0.060	18.5	0.214	0.241
-K	691	3.83	2.96	0.869	0.208	0.074	35.5	0.223	0.364
F.M.	551	3.54	2.73	0.812	0.245	0.088	35.9	0.262	0.217
L. 1 -N	556	3.50	2.73	0.766	0.322	0.072	22.3	0.104	0.268
S. 4 -P	585	2.98	2.16	0.823	0.277	0.088	31.9	0.233	0.225
-K	660	3.07	2.21	0.858	0.277	0.095	34.3	0.271	0.215
F.M.	779	6.28	4.52	1.76	0.371	0.080	21.5	1.05	0.253
L. 2 -N	738	5.88	4.53	1.35	0.366	0.076	20.7	0.689	0.221
S. 2 -P	604	4.76	3.52	1.24	0.362	0.061	16.7	0.666	0.150
-K	824	5.14	3.80	1.34	0.373	0.075	20.2	0.649	0.245
F.M.	864	5.01	3.74	1.27	0.349	0.066	18.8	0.688	0.166
L. 2 -N	799	4.74	3.76	0.984	0.352	0.066	18.8	0.308	0.258
S. 3 -P	851	5.12	3.84	1.28	0.365	0.078	21.2	0.654	0.187
-K	895	5.40	4.00	1.40	0.379	0.083	21.9	0.648	0.293
F.M.	676	4.13	3.02	1.11	0.328	0.099	30.2	0.535	0.152
L. 2 -N	708	3.52	2.61	0.914	0.289	0.079	27.4	0.232	0.314
S. 4 -P	669	3.33	2.32	1.01	0.312	0.081	25.8	0.509	0.105
-K	752	3.76	2.65	1.11	0.248	0.083	33.3	0.610	0.164
F.M.	942	4.03	2.80	1.23	0.333	0.051	15.2	0.712	0.136
L. 3 -N	888	4.82	3.92	0.902	0.320	0.039	12.0	0.421	0.122
S. 3 -P	906	5.19	3.97	1.22	0.347	0.038	11.1	0.675	0.164
-K	976	5.51	4.22	1.29	0.391	0.064	16.4	0.713	0.117
F.M.	700	4.36	3.12	1.24	0.393	0.082	20.8	0.535	0.226
L. 3 -N	713	3.77	2.81	0.955	0.375	0.066	17.5	0.275	0.239
S. 4 -P	684	4.04	2.95	1.09	0.362	0.081	22.2	0.657	—
-K	745	4.53	3.25	1.28	0.391	0.077	19.6	0.671	0.138
F.M.	590	3.66	2.85	0.805	0.266	0.081	30.3	0.362	0.096
L. 3 -N	780	2.70	2.02	0.681	0.221	0.072	32.6	0.087	0.301
S. 5 -P	558	2.93	1.96	0.968	0.247	0.139	56.1	0.385	0.197
-K	725	2.07	1.30	0.768	0.174	0.094	53.9	0.298	0.202
F.M.	625	4.49	3.50	0.992	0.339	0.051	14.9	0.456	0.146
L. 4 -N	606	4.06	3.34	0.723	0.304	0.040	13.1	0.157	0.222
S. 4 -P	620	4.19	3.19	1.00	0.306	0.042	13.9	0.444	0.208
-K	694	4.32	3.33	0.989	0.329	0.050	15.2	0.429	0.181
F.M.	601	4.40	3.47	0.934	0.288	0.056	19.3	0.448	0.142
L. 4 -N	602	2.87	2.18	0.692	0.255	0.051	19.8	0.029	0.357
S. 5 -P	537	3.36	2.21	1.15	0.413	0.165	39.9	0.395	0.180
-K	616	3.65	2.73	0.924	0.322	0.072	22.3	0.289	0.241

TABLE II (continued).

Leaf, sample, and treatment.	Water.	Total N.	Protein N.	Crystal- loid N.	Total amino N.	Amide N.	Amide as % amino N.	Nitrate N.	Residual crystal- loid N.
F.M.	654	4.60	3.75	0.853	0.248	0.046	18.3	0.479	0.080
L. 5 - N	517	2.32	1.89	0.432	0.168	0.033	19.6	0.040	0.191
S. 5 - P	592	3.79	2.71	1.08	0.384	0.169	44.1	0.498	0.033
- K	645	4.16	3.27	0.887	0.303	0.065	21.6	0.296	0.223
F.M.	549	2.93	2.41	0.522	0.193	0.061	31.8	0.136	0.132
L. 5 - N	511	0.89	0.62	0.268	0.095	0.035	36.8	0.009	0.129
S. 6 - P	426	2.12	1.22	0.898	0.332	0.183	55.2	0.184	0.199
- K	410	1.54	0.99	0.551	0.115	0.076	66.4	0.183	0.177
F.M.	623	4.68	3.84	0.844	0.251	0.038	15.1	0.404	0.151
L. 6 - N	488	1.89	1.58	0.309	0.134	0.024	17.6	0.042	0.109
S. 5 - P	700	3.43	2.45	0.976	0.331	0.103	31.0	0.418	0.124
- K	772	4.61	3.67	0.940	0.326	0.052	15.8	0.382	0.180
F.M.	557	3.32	2.73	0.590	0.219	0.041	18.8	0.125	0.205
L. 6 - N	386	1.04	0.82	0.224	0.097	0.018	18.4	0.009	0.100
S. 6 - P	523	1.51	0.82	0.693	0.209	0.114	54.6	0.135	0.235
- K	536	2.99	2.25	0.739	0.257	0.070	27.1	0.265	0.147
F.M.	511	2.61	2.07	0.535	0.205	0.036	17.3	0.074	0.220
L. 6 - N	441	0.62	0.39	0.226	0.058	0.024	40.5	0.012	0.132
S. 7 - P	389	1.23	0.58	0.654	0.178	0.119	66.6	0.188	0.169
- K	—	—	—	—	—	—	—	—	—
F.M.	527	3.38	2.89	0.493	0.184	0.039	21.2	0.092	0.178
L. 7 - N	309	1.08	0.83	0.249	0.074	0.021	28.8	0.014	0.140
S. 6 - P	481	2.90	1.98	0.916	0.398	0.191	47.9	0.252	0.075
- K	573	3.32	2.48	0.838	0.285	0.079	27.8	0.215	0.259
F.M.	484	3.20	2.64	0.558	0.221	0.036	16.2	0.099	0.202
L. 7 - N	339	0.90	0.71	0.190	0.072	0.015	20.6	0.011	0.092
S. 7 - P	444	1.81	0.91	0.902	0.369	0.185	50.1	0.114	0.234
- K	629	2.07	1.16	0.907	0.267	0.106	39.9	0.372	0.162
F.M.	329	1.74	1.25	0.488	0.138	0.054	39.3	0.030	0.266
L. 7 - N	343	0.65	0.43	0.218	0.046	0.022	48.7	0.011	0.139
S. 8 - P	—	—	—	—	—	—	—	—	—
- K	—	—	—	—	—	—	—	—	—
F.M.	452	3.05	2.61	0.444	0.189	0.035	18.3	0.043	0.177
L. 8 - N	321	1.26	1.00	0.259	0.088	0.018	20.5	0.016	0.137
S. 6 - P	444	3.14	2.42	0.719	0.298	0.095	31.9	0.157	0.169
- K	529	3.62	2.86	0.755	0.298	0.058	19.4	0.126	0.273
F.M.	434	3.45	2.88	0.567	0.256	0.038	14.7	0.047	0.226
L. 8 - N	293	1.32	1.08	0.243	0.113	0.016	13.7	0.016	0.098
S. 7 - P	423	3.28	2.15	1.13	0.498	0.263	52.8	0.142	0.229
- K	656	2.88	1.67	1.21	0.489	0.133	27.1	0.262	0.326
F.M.	371	2.64	2.19	0.453	0.162	0.038	23.7	0.038	0.215
L. 8 - N	326	1.48	1.11	0.372	0.104	0.034	32.7	0.016	0.218
S. 8 - P	275	2.17	1.12	1.05	0.428	0.216	50.5	0.109	0.292
- K	—	—	—	—	—	—	—	—	—
F.M.	401	2.93	2.41	0.518	0.210	0.035	16.6	0.033	0.240
L. 9 - N	284	1.53	1.28	0.248	0.109	0.018	16.1	0.010	0.111
S. 7 - P	390	2.86	2.06	0.801	0.324	0.117	36.0	0.082	0.278
- K	520	3.19	2.30	0.893	0.429	0.092	21.4	0.088	0.284



TABLE II (continued).

Leaf, sample, and treat- ment.	Water.	Total N.	Protein N	Crystal- loid N.	Total amino N.	Amide N.	Amide as % amino N.	Nitrate N.	Residual crystal- loid N.
F.M.	300	3.32	2.81	0.510	0.179	0.033	18.5	0.029	0.269
L. 9 - N	276	1.87	1.49	0.376	0.073	0.036	49.7	0.022	0.245
S. 8 - P	274	3.51	2.04	1.47	0.684	0.350	51.2	0.092	0.342
- K	366	2.92	1.75	1.17	0.455	0.205	45.1	0.169	0.337
F.M.	218	3.24	2.81	0.426	0.136	0.037	27.4	0.038	0.215
L. 10 - N	245	1.87	1.59	0.278	—	—	—	—	—
S. 8 - P	241	3.62	2.65	0.973	0.441	0.223	50.6	0.028	0.281
- K	280	4.11	3.09	1.02	0.410	0.121	29.5	0.056	0.429

highest nitrogen contents may be expected in the earlier leaves; in point of fact in all manurings a maximum for the newly expanded members was attained at Leaf 2 or 3. The fall of nitrogen level in later successive leaves is not continued indefinitely, but under nitrogen deficiency a minimum occurs at Leaf 7 or 8, and in the other series at Leaf 9, Leaf 10 showing a secondary increase. In general the fall with leaf age does not reduce the initial high contents of the first few leaves to a very low level, but its effect becomes more marked in Leaves 5-7, while in 8 and 9 in all but the potassium-starved series the nitrogen content *increases* noticeably for some time after complete expansion. The result of these two tendencies in the last-produced leaves may be that the average nitrogen content of all leaves on the plant late in its life history may be higher than that at a somewhat earlier stage, and such increases have indeed been observed (cf. Crowther (6), and Wagner (43)).

These results indicate that in general throughout the period of the first few leaves nitrogen supply is ample; but Leaves 5-7, although beginning with a fairly high content, are rapidly depleted as they age, the nitrogen travelling to younger parts. At this time the nitrogen entering the plant is insufficient for the needs of the meristems, 'internal starvation' becomes pronounced and tillering ceases. In Leaves 8 and 9 at complete expansion the nitrogen level is therefore low, and the amount coming into the plant has been found to be small at this stage. A secondary supply, however, becomes available as the large lower leaves age and liberate much of their nitrogen, and the latest formed leaves continue to accumulate it for some time after expansion, although finally (cf. fully manured and phosphorus-deficient data of Leaf 8) they in turn yield up much of their nitrogen to the expanding ear. Presumably much the same conditions hold in the latest formed leaves under potassium deficiency, but in no case has a rise in nitrogen been observed here after expansion, no doubt associated with the fact that under this treatment leaves are very short-lived. Samples more closely spaced in time would be needed to demonstrate such a rise in nitrogen content. At the youngest observed stage of Leaves 8-10, and

again in the young ears, the nitrogen content is considerably higher under potassium starvation than with complete nutrients; but in the former case the leaf tips are already dying by the time the bases are fully expanded and consequently different portions of the leaf must attain their maximum nitrogen contents at different times. The average content of the whole leaf may not therefore show the typical changes with age which characterize every part of the leaf in turn. From the magnitude of the observed interactions between leaf age, leaf number, and manurial treatment it is readily seen that a most imperfect picture of the nitrogen relations of leaves is obtained if all those on the plant are bulked in the usual manner; indeed in the case of potassium deficiency cited above it would appear that an inadequate representation may be given even by the analysis of single leaves, and that for a complete picture it may be necessary to deal with portions of leaves.

The main differences due to treatment may be summarized as follows. In general, decreasing nitrogen contents are found in the order, fully manured, potassium, phosphorus, and nitrogen deficient. In *early* stages of the leaf, soon after expansion, there is little difference between the potassium starved and those with complete nutrient, except that in the last three leaves the content of the former appears to be the higher. The phosphorus-deficient series is definitely below the fully manured even at the early stage, and the nitrogen deficient of course much lower still. In all deficient series nitrogen content drops much more rapidly with leaf age than in the fully manured, and is most rapid under potassium starvation; the rate of loss of nitrogen is inversely correlated with length of life of the leaves among the treatments. The first four leaves show comparatively slight differences due to treatment.

(2) *Protein nitrogen.* Total protein nitrogen bears a very constant relationship to total nitrogen in the complete and the nitrogen-deficient series, and in the other two series variations are not of a high order, at least in the newly expanded stage. Expressed as percentage dry weight, therefore, many of the remarks relating to total nitrogen are equally applicable to protein. Thus protein content, at the time of complete expansion, does not continue to fall throughout the succession of leaves, but rises again in the last or last two leaves; while considering protein values obtained in the second determination on each leaf (at a more advanced stage), the minimum content is reached much earlier, at about Leaf 6. This result is again due to the general high protein level of early leaves, a relatively quick and more complete disappearance with age in the middle leaves, and a tendency to rise for a time in Leaves 8 and 9, with the exception, as before, of the potassium-deficient and also, to a lesser extent, of the phosphorus-deficient series. Between treatments, decreasing contents of protein nitrogen are again found in the order fully manured,

TABLE III.

Leaf, sample, and treat- ment.	Protein N.	Crystalloid N.	Total amino N.	Amide N.	Nitrate N.	Residual crystalloid N.
F.M.	80.3	19.7	5.3	1.20	7.9	5.3
L. 1 -N	81.0	19.0	5.5	0.95	5.2	7.4
S. 1 -P	78.3	21.7	5.9	1.08	8.5	6.2
-K	79.7	20.3	5.5	1.26	8.4	5.1
F.M.	79.6	20.4	7.3	2.21	7.2	3.7
L. 1 -N	79.4	20.6	7.2	1.49	5.2	6.7
S. 2 -P	76.5	23.5	8.5	2.14	6.5	6.3
-K	77.1	22.9	7.7	1.65	7.5	6.0
F.M.	81.0	19.0	5.6	1.33	6.1	6.0
L. 1 -N	82.1	17.9	7.3	1.30	3.6	5.7
S. 3 -P	80.6	19.4	7.5	1.39	5.0	5.6
-K	77.3	22.7	5.4	1.93	5.8	9.5
F.M.	77.1	22.9	6.9	2.49	7.4	6.1
L. 1 -N	78.1	21.9	9.2	2.05	3.0	7.7
S. 4 -P	72.4	27.6	9.3	2.96	7.8	7.6
-K	72.1	27.9	9.0	3.10	8.8	7.0
F.M.	72.0	28.0	5.9	1.27	16.8	4.0
L. 2 -N	77.0	23.0	6.2	1.29	11.7	3.8
S. 2 -P	74.0	26.0	7.6	1.27	14.0	3.2
-K	73.9	26.1	7.3	1.46	12.6	4.8
F.M.	74.7	25.3	7.0	1.31	13.7	3.3
L. 2 -N	79.2	20.8	7.4	1.40	6.5	5.4
S. 3 -P	74.9	25.1	7.1	1.51	12.8	3.7
-K	74.0	26.0	7.0	1.54	12.0	5.4
F.M.	73.0	27.0	7.9	2.40	13.0	3.7
L. 2 -N	74.0	26.0	8.2	2.25	6.6	8.9
S. 4 -P	69.8	30.2	9.4	2.42	15.3	3.2
-K	70.6	29.4	6.6	2.20	16.2	4.4
F.M.	69.4	30.6	8.3	1.25	17.7	3.4
L. 3 -N	81.3	18.7	6.6	0.80	8.7	2.5
S. 3 -P	76.4	23.6	6.7	0.74	13.0	3.2
-K	76.7	23.3	7.1	1.17	12.9	2.1
F.M.	71.7	28.3	9.0	1.88	12.3	5.2
L. 3 -N	74.7	25.3	9.9	1.74	7.3	6.3
S. 4 -P	72.9	27.1	9.0	1.99	16.3	—
-K	71.8	28.2	8.6	1.69	14.8	3.0
F.M.	78.0	22.0	7.3	2.20	9.9	2.6
L. 3 -N	74.8	25.2	8.2	2.67	3.2	11.1
S. 5 -P	67.0	33.0	8.4	4.73	13.1	6.7
-K	62.9	37.1	8.4	4.53	14.4	9.8
F.M.	77.9	22.1	7.6	1.13	10.2	3.3
L. 4 -N	82.2	17.8	7.5	0.98	3.9	5.5
S. 4 -P	76.1	23.9	7.3	1.01	10.6	5.0
-K	77.1	22.9	7.6	1.16	9.9	4.2
F.M.	78.8	21.2	6.5	1.26	10.2	3.2
L. 4 -N	75.9	24.1	8.9	1.76	1.0	12.4
S. 5 -P	65.7	34.3	12.3	4.90	11.8	5.4
-K	74.7	25.3	8.8	1.97	7.9	6.6

TABLE III (continued).

Leaf, sample, and treat- ment.	Protein N.	Crystalloid N.	Total amino N.	Amide N.	Nitrate N.	Residual crystalloid N.
F.M.	81.5	18.5	5.4	0.99	10.4	1.7
L. 5 - N	81.4	18.6	7.2	1.42	1.7	8.2
S. 5 - P	71.4	28.6	10.1	4.46	13.1	0.9
- K	78.7	21.3	7.3	1.57	7.1	5.4
F.M.	82.2	17.8	6.6	2.09	4.6	4.5
L. 5 - N	69.9	30.1	10.7	3.93	1.0	14.5
S. 6 - P	57.6	42.4	15.7	8.65	8.7	9.4
- K	64.2	35.8	7.5	4.96	11.9	11.5
F.M.	82.0	18.0	5.4	0.81	8.6	3.2
L. 6 - N	83.7	16.3	7.1	1.25	2.2	5.8
S. 5 - P	71.5	28.5	9.7	2.99	12.2	3.6
- K	79.6	20.4	7.1	1.12	8.3	3.9
F.M.	82.2	17.8	6.6	1.24	3.8	6.2
L. 5 - N	78.5	21.5	9.3	1.71	0.9	9.6
S. 6 - P	54.1	45.9	13.8	7.56	8.9	15.6
- K	75.3	24.7	8.6	2.33	8.9	4.9
F.M.	79.5	20.5	7.9	1.36	2.8	8.4
L. 6 - N	63.5	36.5	9.4	3.79	1.9	21.3
S. 7 - P	46.8	53.2	14.5	9.63	15.3	13.7
- K	—	—	—	—	—	—
F.M.	85.4	14.6	5.4	1.15	2.7	5.3
L. 7 - N	76.9	23.1	6.9	1.97	1.3	13.0
S. 6 - P	68.4	31.6	13.7	6.58	8.7	2.6
- K	74.8	25.2	8.6	2.39	6.5	7.8
F.M.	82.6	17.4	6.9	1.12	3.1	6.3
L. 7 - N	78.9	21.1	8.0	1.64	1.2	10.2
S. 7 - P	50.2	49.8	20.4	10.2	6.3	12.9
- K	56.2	43.8	12.9	5.14	18.0	7.8
F.M.	72.0	28.0	7.9	3.11	1.7	15.3
L. 7 - N	66.5	33.5	7.1	3.45	1.7	21.4
S. 8 - P	—	—	—	—	—	—
- K	—	—	—	—	—	—
F.M.	85.4	14.6	6.2	1.13	1.4	5.8
L. 8 - N	79.4	20.6	7.0	1.43	1.3	10.9
S. 6 - P	77.1	22.9	9.5	3.03	5.0	5.4
- K	79.1	20.9	8.2	1.60	3.5	7.5
F.M.	83.6	16.4	7.4	1.09	1.4	6.6
L. 8 - N	81.6	18.4	8.6	1.17	1.2	7.4
S. 7 - P	65.5	34.5	15.2	8.02	4.3	7.0
- K	58.0	42.0	17.0	4.60	9.1	11.3
F.M.	82.8	17.2	6.1	1.45	1.4	8.1
L. 8 - N	74.9	25.1	7.0	2.30	1.1	14.7
S. 8 - P	51.8	48.2	19.7	9.96	5.0	13.5
- K	—	—	—	—	—	—
F.M.	82.3	17.7	7.2	1.19	1.1	8.2
L. 9 - N	83.8	16.2	7.1	1.15	0.7	7.3
S. 7 - P	72.0	28.0	11.3	4.08	2.9	9.7
- K	72.0	28.0	13.4	2.87	2.8	8.9

TABLE III (*continued*).

Leaf, sample, and treat- ment.	Protein N.	Crystalloid N.	Total amino N.	Amide N.	Nitrate N.	Residual crystalloid N.
F.M.	84.6	15.4	5.4	1.00	0.9	8.1
L. 9 -N	79.9	20.1	3.9	1.94	1.2	13.1
S. 8 -P	58.2	41.8	19.5	9.97	2.6	9.7
-K	60.1	39.9	15.6	7.03	5.8	11.5
F.M.	86.9	13.1	4.2	1.15	1.2	6.6
L. 10 -N	85.1	14.9	—	—	—	—
S. 8 -P	73.1	26.9	12.2	6.16	0.8	7.8
-K	75.3	24.7	10.0	2.94	1.4	10.4

potassium, phosphorus, and nitrogen deficient, though in the early leaf stages soon after full expansion there is no significant difference between the first two. The drop with leaf age is most rapid under potassium starvation, followed by phosphorus deficiency, the fully manured series maintaining its protein longest. This order is the same as that shown in the dying off of the leaves: protein hydrolysis is associated with death.

At the time of the second determinations of Leaves 8 and 9, the phosphorus-starved leaves had a higher protein content than the potassium-starved. It must be remembered that potassium-deficient leaves die considerably earlier than phosphorus-starved, and when leaves are sampled at more or less comparable stages rather than at the same time those from the latter series always appear to show a much lower protein content. Thus in the last-analysed samples of Leaf 8, Sample 7 for potassium deficiency and Sample 8 for phosphorus, the leaves in these two treatments were as nearly comparable as can be judged, approximately one-third of the distal portion being dead and shrivelled in both cases. At these times the protein nitrogen content of the potassium-starved set was 1.67 per cent., and of the phosphorus-starved 1.12 per cent.

In Leaves 4, 6, 8, 9, and 10, at the first stage analysed, the protein content of the fully manured series was never so much as 5 per cent. greater than that of the potassium-deficient series, and in Leaves 8 and 10 was 10 per cent. lower; but in Leaves 5 and 7 it was greater by 13 per cent. and 14 per cent. respectively. In the last-mentioned two leaves the first analysis in reality represents a more advanced stage than that of the other leaves, and the presumption is strong, therefore, that where potassium was lacking, protein was being hydrolysed more rapidly than synthesized and was disappearing from the leaves even at the time of complete expansion. Very possibly this loss had been going on prior to the time of the first sampling of the other leaves, in which case their initial protein content must have been higher than in corresponding leaves from fully manured plants. At any rate, there is no doubt that, initially, potassium-deficient leaves have at least as high total protein contents as those from the fully manured series.

The same considerations do not hold for phosphorus deficiency, in which protein nitrogen was consistently below the fully manured and potassium-deficient values at expansion, even though phosphorus-starved leaves are in fact relatively rather younger than those from the other two treatments, and in the case of Leaves 6 and 9, were not completely expanded at the time of sampling. With the exception of Leaf 6, which was markedly affected by the previously mentioned crisis in the history of these plants, the rate of loss of protein was in general less rapid than under potassium deficiency. Hence it is safe to assert that in their youngest stages phosphorus-starved leaves do not have so high a protein content as either potassium-starved or fully manured, and that protein synthesis in meristematic tissue is definitely slower than with these other two treatments.

(3) *Total amino-nitrogen.* In the case of total amino-nitrogen considerable interactions between treatment, leaf number, and leaf age were found. The newly emerged leaves of all the four series showed a sharp rise from the first to the second; at this early stage of the plant manurial differences were not yet apparent. In the fully manured series a high value was maintained until Leaf 4, after which the successive leaves showed decreasing amino content. The change in any particular leaf with age is not very clear, but it again appears that there was a marked rise for some time after expansion in the later leaves, and in all leaves an eventual fall to a value considerably below the initial value. In the first three leaves it is impossible to say definitely whether or not a rise occurred after expansion, owing to these suffering to a certain extent from iron deficiency, and the rise observed in Leaves 1 and 3 may be due to this cause. Ignoring these, there is no indication of any rise during the life of the leaf until Leaf 7 is reached, where one of 20 per cent. was observed. In Leaf 8 a very considerable rise of 36 per cent. was found, but in 9 again no rise was observed, doubtless because of the fourteen-day interval between the two samplings, a time amply sufficient for a rise and subsequent fall to have occurred.

In general, therefore, it follows that there is a marked parallelism between the variations in protein and amino-acid content in the successive leaves and also throughout the history of individual leaves; but it should be remarked also that in the data evidence can be found of an inverse correlation between these two variables during the history of individual leaves, when the variables are expressed as percentage total nitrogen. These two correlations are in accord with the views expressed later as to the supply of nitrogenous compounds to the various leaves, and the increasing rate of protein hydrolysis compared with synthesis that occurs with leaf age.

Under nitrogen deficiency the total amino-nitrogen contents vary in a very similar manner to those of the fully manured series, with the difference that after Leaf 4 they are at a considerably lower level.

Under phosphorus and potassium deficiency the increase noticed by other workers is amply confirmed. Under the particular nutrient conditions of the experiment this was not noticeable in the first four leaves soon after expansion, but as the values in the fully manured and nitrogen-starved series rapidly declined in later leaves, those in the phosphorus- and potassium-starved series remained almost as high as in the earliest leaves. A slight drop to a minimum value at Leaf 7 or 8 occurred under potassium deficiency, and a similar minimum was found at 8 under phosphorus deficiency. Leaves 9 and 10 again showed a large rise and provided the highest figures of any determinations at so early a stage in individual leaf history. The accumulation of amino-nitrogen occurred very suddenly under phosphorus deficiency: at Sample 4 there was no indication of abnormality in amino-acid content in any of the leaves present, but at the next sample all active leaves were affected, Leaf 6 being 32 per cent. above its control, Leaf 5, 55 per cent., and Leaf 4, 43 per cent. Between these two samples the appearance of the plants changed markedly. With this manuring the onset of deficiency symptoms affects not only the new parts, but also those leaves which are already mature (cf. Leaf 4 with 0.306 per cent. at Sample 4 and 0.413 per cent. at Sample 5). Strong indirect evidence is therefore afforded that phosphorus, like nitrogen and potassium, is translocated from old leaves to be re-utilized at the meristem. After the change to a relatively higher total amino-nitrogen level in this series a drop with leaf age was observed in Leaves 5-7, but in Leaf 8 a considerable rise of 67 per cent. was found after expansion, presumably owing to the supply from dying leaves below; while in Leaf 9 the rise after expansion was from 0.324 per cent. to 0.684 per cent., this last being much the highest amino content found in this experiment. That this must have been due to supply from sources external to the particular leaf is almost certain, for protein nitrogen was unchanged, while in the corresponding leaf of the fully manured and nitrogen-deficient series protein rose while amino-nitrogen fell.

Under potassium deficiency rises are again found: in Leaf 8, 64 per cent., and in Leaf 9, 6 per cent. The time interval corresponding to the 6 per cent. rise in Leaf 9 was large, and at Sample 8 the leaves were rapidly dying; doubtless there had been a very considerable rise in amino-nitrogen with a subsequent fall during the time between these two samples.

(4) *Amide-nitrogen.* In the fully manured series a marked rise in amide-nitrogen as percentage dry weight, as in all other fractions, was found from Leaf 1-2. Thereafter a sharp fall occurred until in Leaf 6 and all subsequent ones the level was below 50 per cent. of this maximum value. Later leaves showed little change, though there is again evidence of a minimum at Leaf 8 or 9. The change in concentration with leaf age is rather irregular, but in general there appears to be a tendency to rise, while in terms of percentage total nitrogen the rise as leaves age is very

real. The changes induced by nitrogen starvation are difficult to assess ; in general a lower concentration is found on a dry weight basis, and a higher on a total nitrogen basis. The rise with leaf age, too, is more marked than in high-nitrogen plants.

The phosphorus- and potassium-deficient series gave much increased amounts of amide, particularly the former. In both, the amount increased relative to the fully manured series between Samples 4 and 5, and affected all leaves living at the time. The marked fall in amide content of the young leaves found in the controls between Leaves 2 and 6 was largely eliminated under potassium starvation, while the rise with leaf age was considerably accentuated. Under phosphorus starvation the increase in all leaves between Samples 4 and 5 was relatively very great, and throughout the later life of the plants amide content was maintained at a very high level. In the older leaves approximately 10 per cent. of the total nitrogen present may be as amide, and probably therefore nearly 20 per cent. exists as asparagine and possibly glutamine. This very high concentration is perhaps the most characteristic of all the changes induced in the nitrogen fractions by lack of phosphorus.

Changes due to manurial differences in amide-nitrogen expressed as a percentage of total amino-nitrogen are also of interest. On the assumption that all the amide exists in the form of asparagine, this figure is equivalent to the proportion of the total amino-nitrogen accounted for by the amino group of asparagine, since half the nitrogen of this substance is estimated as amide- and half as amino-nitrogen. In the fully manured series the ratio rises with leaf age, and particularly so in dying leaves. It is not affected much by nitrogen starvation, although the rise with leaf age appears to be more rapid. In the phosphorus-starved series, after the fourth sample, the value is much above normal even in the early leaf stages, and continues to rise with age.

Under potassium starvation, on the other hand, in the early leaf stages the values are approximately equal to those of the fully manured series ; young leaves, therefore, have almost the same ratio of asparagine to amino acids in the two series, although there is a much more rapid rise with age under deficiency. But the rise needs interpreting in relation to 'physiological' or 'metabolic' age rather than to absolute age, since on deficient plants the leaves die much sooner. Table I shows that in the later samples, when the symptoms of deficiency were pronounced, the second analysis of potassium-starved leaves always occurred when these were rapidly dying or largely dead, whereas at the same time fully manured leaves were wholly green. In Leaves 6-8 of the latter series a third sample was possible, but in these also, with one exception, only the leaf tips were obviously affected. The exception is Leaf 7 at Sample 8, which was half dead, while Leaf 7 of the potassium-deficient series was in approximately the same state at Sample 7.



At this stage both sets had the same ratio value, 39.3 and 39.9 respectively ; there is a strong presumption, therefore, that in their final stages the control leaves attain values somewhat similar to those of the deficient series. Under nitrogen starvation, too, which in these experiments has produced little deviation from the normal in the relative distribution of the various fractions, the leaves age somewhat more rapidly than with high nitrogen, and in three cases (Sample 6, Leaf 5 ; Sample 7, Leaf 6 ; and Sample 8, Leaf 7) the leaves were at least half dead, while the ratio attained high values similar to those in all other rapidly dying samples.

It therefore appears normal for leaves as they age to convert much of their soluble nitrogen into amide, in which form it is possible that most of the translocation of nitrogen occurs. Under potassium starvation the relation between the two forms of nitrogen at any one 'physiological stage' appears, therefore, to be nearly normal. The argument previously used, based on the relatively advanced age of Leaves 5 and 7 at the time of their first sampling, to demonstrate that protein content is at least as high in young leaves under potassium deficiency as under high potassium, applies with equal force in the case of the ratio of amide- to amino-nitrogen.

The very high value found in Leaf 5 of the potassium-deficient series at Sample 6 needs some comment. Although the sample was but two-thirds dead as judged by withering, yet it was probably the most senescent of all the experimental leaves. The water content had the very low value for potassium deficiency at this stage of 41.0, while most of the nitrogen had been evacuated ; total nitrogen had fallen from 4.16 per cent. at the previous sampling date to 1.54 per cent., and protein had dropped correspondingly from 3.27 per cent. to 0.99 per cent., these final values being much the lowest of any recorded in the series.

(5) *Nitrate*. The nitrogen was applied to the pots previous to the time of the first sample, and the rapid uptake in early stages is reflected in a large increase of nitrate, as well as all forms of organic nitrogen, from Leaf 1 to Leaves 2 and 3, in the last two nitrate being maximal. The very high value in the fully manured series, Leaf 2, Sample 2, is probably exaggerated by the iron deficiency which appeared as a chlorosis of the second and third leaves between Samples 2 and 3, and resulted in disappearance or reduced production of protein in these leaves. Nitrate content in this series declines with the successively later leaves, a large drop occurring between Samples 5 and 6. Up to this stage tillering has been active, but it now ceases with the failure of the supply of nitrogen, and probably other nutrients. Traces of nitrate are found even in the tenth leaf. In the earlier leaves, where nitrate is abundant, it decreases rapidly as they age, but death apparently occurs with considerable amounts of unused nitrate still present.

Under nitrogen deficiency precisely similar relations are found, but

here the drop with leaf number is earlier and the depletion with leaf age more pronounced. In both the phosphorus- and potassium-deficient series some accumulation of nitrate occurs in later leaves; in the last three samples considerably greater amounts are found in these series than in the control, this being particularly noticeable in Leaves 7 and 8. It is interesting to note that in these two leaves, whereas the nitrate level is originally higher under phosphorus starvation, it rapidly decreases with leaf age, while under potassium deficiency it increases markedly so that the content is eventually two or three times as great as in the former series. Whether this is due mainly to differences in reductase activity of the two types or to the balance between nitrate supply and its removal it is impossible to decide from the data.

#### DISCUSSION.

In reviewing the manurial results which have been presented it may be well to indicate firstly the direct causes of the reduction in dry weight of the various plant types analysed. In the case of potassium deficiency this is primarily due to two factors: (1) reduction in leaf area brought about almost entirely by the very early death of leaves, and (2) a much reduced photosynthetic activity of the living leaves. Under nitrogen deficiency photosynthetic activity is approximately normal, but leaf area is much reduced; this is not so much due to reduction in the length of life of leaves as to lowered meristematic activity, so that the area of individual leaves is small. Moreover the total number of leaves produced is diminished owing to a much reduced tillering rate. Finally, in the case of phosphorus deficiency all the above factors appear to be operative to a high degree: leaf area is reduced as a result of reduction in size and length of life of individual leaves, and also of a lowered tillering rate, while photosynthetic rate is also reduced at the present level of manuring (unpublished results by R. F. Jones working at this Institute). In a previous communication (11) it was shown that under a moderate degree of phosphorus starvation photosynthesis is not appreciably affected.

(i) *Effect of nitrogen deficiency.* Deficiency of nitrogen has not resulted in any striking departures from the normal in the distribution of the organic fractions estimated. Expressed in terms of percentage total nitrogen the agreement between the two series is in general close, although in terms of dry weight the whole nitrogen level is of course much reduced under starvation. Suggestions of differences do exist, but the evidence for them is not sufficiently strong to warrant a discussion of their probable significance. Nitrate, as might be expected, drops with the age of the plant much more quickly than in the other series. On the other hand the unestimated crystalloid nitrogen appears to be present in relatively greater concentration than the estimated fractions.

According to Engel (10) leaves from nitrogen-starved plants have a very reduced ratio of amide- to amino-nitrogen, and a similar claim is made by Webster (44) for all parts of the soy bean. But if the actual values are calculated from Webster's data for green leaves the ratio is higher under starvation at four samples and lower at only three; the mean ratio is 0.218 under deficiency and 0.203 in high-nitrogen leaves. Engel believes that since under these conditions nitrogen for growth must be obtained from previously utilized nitrogen there is an active hydrolysis of protein in the mature organs. Carbohydrates are abundant, and in consequence the resulting amino acids are not deaminated with the formation of asparagine *in situ*, but are first translocated to the growing regions. The present results do not confirm this view. After the fourth leaf there appears to be very little difference between the two series. As has been mentioned, in Leaves 2 and 3 of the fully manured series a certain amount of iron chlorosis occurred. This was accompanied by reduced protein content, and presumably by reduced protein synthesis, so that the normal relationship between amide- and amino-nitrogen may have been disturbed. In spite of this the figures do suggest that in these early stages of deficiency the ratio of amide- to amino-nitrogen may indeed be slightly reduced; if so, the deviation is much less than Engel apparently found. But it is in the later stages that nitrogen starvation becomes acute, and here the value of the ratio is approximately normal. It should be pointed out however that the analyses in the case of nitrogen-deficient leaves were difficult and comparatively large errors may be involved in later leaves; but the results do indicate strongly that under nitrogen starvation the distribution of the fractions, and presumably therefore the course of metabolism, is very little different from normal.

A partial explanation of this divergence of results is that Engel did not estimate amino-nitrogen directly, but from the difference between total soluble nitrogen and the sum of ammonia and amide-nitrogen. The 'residual crystalloid' nitrogen of this investigation is therefore included by him as amino-nitrogen, and, as is stated above, the former fraction is found in comparatively high concentration under nitrogen starvation, so that in these experiments the ratio of amide to amino *plus* residual nitrogen is likewise reduced. But there appears to be also a real difference between Engel's results and the present ones in that under deficiency he found a considerably reduced amount of amide relative to total soluble nitrogen.

It is nevertheless true that leaves of a low-nitrogen plant are not so long-lived as those of a high-nitrogen plant. Engel apparently believes that in spite of high carbohydrate there is an obligate break-down of protein in mature leaves in order to supply the growing point with materials necessary for synthesis. In this connexion two possible considerations

may be put forward; (1) since a nitrogen-starved leaf has from the start a much reduced protein content it is probable that less hydrolysis need occur, and a shorter time elapse, before the protein level becomes so low as to lead to death; and (2) since the growing region is itself starved of nitrogen the tendency of soluble nitrogenous compounds to migrate to the meristems may be greater than in a high-nitrogen plant, i.e. protein decomposition products liberated by the normal hydrolysis within the leaf may tend to be translocated away more rapidly than in a fully manured plant, though this effect will be counterbalanced at least partly by the reduced concentration of these substances within the leaf.

(ii) *Effect of phosphorus deficiency.* In the case of phosphorus deficiency it is evident that protein synthesis is seriously impaired. The protein contents are consistently below those of the fully manured and the potassium-deficient series when the leaves are young, and it is safe to assert that synthesis in meristematic tissue is definitely reduced, being dependent on the presence of a sufficient concentration of phosphorus. In consequence there is a large accumulation of amino- and particularly of amide-nitrogen. That protein synthesis should be slowed down under deficiency of phosphorus is not surprising considering the importance to cells of the nucleoproteins, but it is impossible to state from the present results whether synthesis of the simpler proteins which are unconnected with phosphorus is likewise directly dependent on the presence of the element. According to MacGillivray (21) under deficiency most of the phosphorus is found at the apical meristems, while Eckerson (8) records that phosphorus remains for a long time in or near a secondary cambium, i.e. it is found in those places where protein synthesis is most pronounced.

The results reported by MacGillivray are somewhat conflicting. Leaf protein (obtained from his figures by the addition of insoluble and coagulable nitrogen) was in one experiment not affected by deficiency, in another it was considerably increased, while in a third it was just as considerably reduced. Similar data for stems and fruits are equally conflicting. Crystalloid nitrogen was always much increased by deficiency, and nitrate in general accumulated as in the present experiments. Kraybill and Smith (17 and 18) state that they have obtained reduced protein contents and increased soluble nitrogen and nitrate in tomatoes under phosphorus deficiency, but their figures do not appear to be available, while Eckerson (8) came to the conclusion that the first symptoms of deficiency are due primarily to loss of reductase activity. This she believes results in internal nitrogen starvation even when nitrate occurs in considerable concentration within the plant. From the results presented here it appears that up to the time of the fourth sample the distribution of the nitrogen fractions was normal, but by the fifth protein had dropped, amide- and amino-nitrogen had increased in all leaves very markedly, while nitrate

was yet but little above normal. Reducase inactivity cannot therefore have been the first symptom of disorganization of the nitrogen metabolism, but rather an inability to build up complex molecules from the simpler organic nitrogenous bodies.

Under the circumstances of the experiment the onset of symptoms of protein derangement was sudden, as may be seen from the marked changes which occurred between Samples 4 and 5. The most striking feature of these analyses is the sudden rise of amide to a very high level in all the living leaves, a level which is maintained throughout the subsequent life of the plant. Amino acids generally are increased, but it is the accumulation of amide, and presumably asparagine that is the most characteristic feature of phosphorus starvation. Work by Gregory and Baptiste now in the press shows that at precisely the same time there is a sudden and large increase in reducing sugars. The two types of accumulation may be intimately connected and possibly represent the simple substances from which protein is normally synthesized.

Correlated with reduced rate of protein synthesis, and presumably dependent on it, are a marked reduction in leaf size and a more tardy rate of leaf production. The slowing down of tiller production may also be a direct effect of the inability to form new centres of protein production, since the available phosphorus is entirely located in existing growing regions. All these features are also characteristic of nitrogen starvation, where almost certainly low protein concentration is the causal factor in reduced vegetative development.

(iii) *Effect of potassium deficiency.* It is commonly stated in the literature that potassium is essential for, and plays a direct part in, the synthesis of proteins in plants, a view which has been supported by many recent workers (2, 14, 41) and particularly by Nightingale, Schermerhorn, and Robbins (25). The belief is based mainly on the following facts:—(1) in potassium deficiency there is an accumulation of amino acids and asparagine; (2) as usually described, such plants do not produce new meristems freely and growth rate and size are reduced, so therefore the total amount of protein produced is subnormal; (3) potassium accumulates mainly at the centres of active protein synthesis, chiefly in the meristems but also to a considerable extent in leaves. The first consideration might be expected to lead to the conclusion that under potassium starvation the percentage of protein in the tissue would be lowered, but analysis of such tissue usually indicates normal, or even supernormal amounts of protein. To explain this fact Nightingale *et al.* discriminate between storage proteins and proteins of the living protoplasm and imply that it is only the latter which depend on potassium for their synthesis, so that reduction in protoplasmic protein is more or less balanced by excess of storage protein.

From their results this hypothesis appears plausible since in the early

stages of deficiency carbohydrate accumulation occurred, a condition which they believe to be especially favourable to synthesis of storage protein.

The fact that potassium is found in high concentration at the meristems does not necessarily imply its part in protein synthesis, and indeed Reed (33) believed that it is in some manner necessary for cell, and particularly for nuclear, division. He too thought it possible that the reason the elongated nuclei remained undivided in his experiments is that potassium is concerned in the elaboration of nucleoproteins. Macallum (20), however, came to the conclusion that the nucleus is completely free from potassium, and later workers (19, 29, 45) have similarly failed to detect its presence there; if it is absent it follows that any protein within the nucleus dependent on the presence of potassium for its synthesis must have been elaborated outside the structure and translocated to it, a hypothesis which is not very plausible.

Even should potassium be necessary for protein synthesis in the meristem it is very possible that it may still proceed there normally even where there is deficiency of the element in the plant as a whole, for a large part of the potassium is in conditions of starvation found accumulated at the meristems. From analyses of total protein reported here it appears that protein synthesis proceeds at the normal rate in the young tissues. The total protein content in young leaves is at least as high as in those of fully manured plants, while the leaves are large and not markedly reduced in size as under nitrogen and phosphorus starvation; moreover, the rate of leaf production is approximately the same as in a fully manured plant, while under the other two types of deficiency reduction is shown. Similar high protein contents have been found by other workers; this result may presumably be taken as general. But in elucidating the results here presented the arguments of Nightingale *et al.* concerning the distinction between storage and protoplasmic proteins cannot well be applied, for in the potassium-deficient plants here described carbohydrate concentration is considerably reduced throughout the life history, and there is at no time a high carbohydrate phase as the results of Gregory and Baptiste show. If high carbohydrate led to the formation of larger amounts of storage protein as Nightingale claimed, one must also admit the probability that the reduced carbohydrates in the present experiments will have led to a subnormal content of storage protein. Hence, since total protein in the young leaves was normal in amount, the protoplasmic proteins must have been supranormal, and their synthesis in the meristems cannot have been adversely affected by the deficiency of potassium.

Exactly similar effects on the nitrogen fractions of young leaves are thus obtained under potassium deficiency whether carbohydrates are subnormal or supranormal in amount, and the effect of carbohydrate concentration within the plant cannot therefore be of great importance.

On the other hand, Nightingale *et al.* are certainly correct in ascribing

the early accumulation of carbohydrate they observed to a reduction of total amount of protein synthesis within the plant. This is largely explained by failure to produce new growing points, a characteristic of the plants they used. Why new meristems should not have appeared is obscure, but others have obtained similar results: Hartt (13) for instance found the number of tillers in sugar cane reduced by approximately 75 per cent. after eleven weeks of potassium-free treatment, while probably, as in Janssen and Bartholomew's work (16), wherever high carbohydrate is reported at the same time as high crystalloid nitrogen, protein nitrogen being normal, it may be inferred that the plant investigated belongs to this type. No marked reduction in meristem production was found by the present authors in the barley under discussion, nor with three species of grasses similarly treated. With the type of potassium deficiency here studied tillering is free and carbohydrate concentration is thereby reduced, as compared with that observed by many workers on potassium deficiency. Also in this experiment there is a reduction in carbohydrate as compared with the fully manured plants; this is accounted for by reduction in photosynthesis (11, 34). The contrast between potassium deficient plants and those under nitrogen or phosphorus deficiency with their greatly reduced tillering and high carbohydrate is most striking from the time the first symptoms develop. The problem of meristematic activity and carbohydrate level is therefore not directly one of potassium supply, but represents an interaction effect between potassium and the other ions in the nutrient solutions, an interaction similar to those reported in a previous paper (34). Experiments with a view to elucidating the effect of balance of ions are now being carried out at this Institute.

The distinction between the two types of plant found under potassium deficiency, caused by variations in the relative concentrations of other ions, cannot be too strongly emphasized. Only such effects may be directly attributed to the potassium ion which are common to all conditions in which potassium concentration is limiting growth; other effects which vary with the general type of nutritive solution must be largely secondary and represent interactions of the element with other ions, i.e. different types of physiological unbalance, antagonism and so forth. In this category must be placed carbohydrate concentration, chlorophyll, and anthocyanin or phloroglucin (cf. 13) production, water content and rate of production of new meristems, this last being possibly one aspect of a more general change in capacity for cell division (cf. 33). Because of the widely divergent types of plant obtainable under reduced potassium, great caution is necessary in the interpretation of results and in the attempt to correlate the results of different workers. Somewhat similar interactions, due to the general type of nutritive solution, may possibly be found in the case of other deficiencies, but it is unlikely that they are of a magnitude as great as under potassium starvation. A possible instance may be found in total nitrogen content under phosphorus deficiency.

Kraybill and Smith (17, 18) report an increased nitrogen content and MacGillivray's results with tomato (21) are similar, except in the case of the youngest leaves; on the other hand Thomas (39) found a considerable decrease in apple trees under deficiency, just as in the present case of barley leaves.

Though under potassium deficiency protein synthesis in meristematic leaf tissue may be presumed therefore to proceed at the normal rate, in the present experiments the protein level was not maintained in the mature leaves as in those of a fully manured plant; the content dropped rapidly with time, the fall being accompanied by early death. If it be true that potassium is essential to some stage in protein synthesis in mature leaves it follows from the above discussion that the rapid loss of protein must presumably be ascribed to early disappearance of potassium from these organs. Early deterioration and death of leaves, often referred to as 'die-back', appears to be a universal symptom of potassium starvation. *There can be no doubt that this rapid hydrolysis is the main source of the high amino acid and amide contents in potassium-deficient plants*, and that these compounds are not largely primary synthetic substances. Nightingale *et al.* held that the accumulation of simpler nitrogenous substances throughout the plant 'may indicate the necessity of potassium in synthesis of polypeptides', while Hartt (12) in 1929 reported decreased peptase activity under deficiency of the element, though in later work (14) she was unable to detect the enzyme even in 'high-potassium' plants. Burrell (2) too believed that potassium was essential to synthesis, and somewhat similar views have been expressed by Turtshchin (41), who is of the opinion that potassium is connected in some way with the dehydration or polymerization of the simpler nitrogenous and carbohydrate substances. Schmalfuss (35, 36) on the other hand argued that increased proportions of soluble nitrogenous compounds and simpler carbohydrates are not likely to be linked with any direct action of the potassium ion. In this connexion (36) he draws a parallel between wilting leaves and those of deficient plants with a low water content, a parallel which, however, loses much of its cogency in the experiments here described, where the water content is very considerably above normal. Finally, Phillips, Smith, and Dearborn (30), who studied the onset of symptoms in deficiency, found no evidence of deranged nitrogen metabolism. They concluded that there is no reason for assuming difficulty in protein synthesis in the earlier stages, but made no distinction between storage and protoplasmic proteins. At this time the weight and height of their plants were definitely reduced and there was apparently considerable reduction in leaf length; significant deviations from normality also occurred in the carbohydrate fractions and in water content.

The following explanation of the relation between the various nitrogen fractions under deficiency of potassium, appears to the authors to correlate



the known facts better than does the hypothesis suggested by Nightingale *et al.*: Potassium, though not directly concerned in protein synthesis, plays a part in maintaining some essential process or constituent of the protoplasmic complex, and possibly in the building of some non-proteinaceous protoplasmic substance. It may be that it is directly concerned in nitrogen metabolism in determining the rate of protein hydrolysis, but it appears more probable that its effect on the distribution of nitrogen fractions is entirely indirect and due to its necessity for some more subtle process essential to protoplasmic maintenance. Under deficiency of the element, therefore, although protein may be continually produced in living leaves, as a result of general protoplasmic injury and deterioration it is broken down much more rapidly than synthesized. Hydrolysis is due to premature ageing and death rather than *vice versa*. The resulting increased concentration of the simpler nitrogenous compounds throughout the plant may itself conceivably lead, in young parts, to an *increase* in rate of protein synthesis rather than a decrease so long as protoplasmic degeneration is not advanced, but with age the balance definitely moves in the direction of rapid hydrolysis and disappearance of protein. This hypothesis meets equally well all the facts that fit the assumption that potassium is necessary to protein synthesis, and is in agreement with certain others that do not fit so well into Nightingale's theory. The following three considerations will make this clear.

(1) The results of Phillips *et al.* (30), referred to above, demonstrate that in early stages of deficiency no deviations from normal in the nitrogen fractions are found; the authors concluded that in these stages there is no reason for assuming synthesis to be dependent on potassium. At the time all other usual symptoms of deficiency had appeared, and whereas the leaves of control plants were healthy, all but the youngest in the deficient series had developed light brown spots, while the lowest were affected more seriously, and a few leaflets were dead. If an inability to synthesize protoplasmic proteins is, as Nightingale believes, the primary cause of reduction in growth and of the high concentration of amino-nitrogen in the plant, amino acid accumulation would be one of the first symptoms of deficiency and not, as appears from Phillips's results, one of the last. It is, moreover, clear that the reduced growth rate together with rapid deterioration of older leaves observed by Phillips *must inevitably lead to the accumulation of protein degradation products*.

(2) In this relationship certain respiration experiments may be considered. It was held by Palladin (27), from experiments with wheat seedlings germinating in darkness, that so long as carbohydrate supply is adequate the rate of respiration is proportional to the amount of protein not digested by gastric juice, this being taken as a rough measure of the protoplasmic proteins. He believed (28) that in general respiration rate varies with the mass of protoplasm, since it is directly caused by enzymes

whose amount is dependent on the amount of protoplasm producing them. Some such relationship certainly holds, and work done in this Institute shows conclusively that in highly manured plants the correlation between total protein and respiration rate in leaves is always very high. Unpublished work by Gregory and Sen shows that under nitrogen starvation a similar relation holds, and that the rate of respiration per unit of protein nitrogen increases with the degree of deficiency, although on a leaf dry weight basis it decreases (11). It seems reasonable to suppose therefore that under deficiency of nitrogen, in spite of a very high carbohydrate content, the nitrogen of the leaves at least is used in the formation of protoplasmic proteins and storage protein is probably relatively reduced in amount. Nightingale *et al.* (24), on the contrary, came to the somewhat surprising conclusion that in tomato under nitrogen starvation nitrogen is locked up as storage protein in considerably supranormal amounts.<sup>1</sup> Under potassium deficiency it has been shown (11, 34) that the respiration rate of young leaves on a dry weight basis is considerably increased in spite of a much reduced potassium content. The results of Gregory and Sen, and the present protein figures, considered in relation to respiration values obtained from leaves of similarly treated plants (34), show that on a basis of protein nitrogen respiration is similarly supranormal, though not so high as under nitrogen deficiency. It is, therefore, unlikely that much of the protein is of a storage nature. Even assuming that there is as much storage protein as in fully manured plants, the protoplasm has an inordinately high respiratory activity.

(3) A further consideration relates to the amino acid and amide fractions. The main manurial effects on these fractions may be summarized as follows: in both potassium and phosphorus deficiencies amide- and amino-nitrogen accumulate, but, while under phosphorus starvation the ratio of amide- to total amino-nitrogen is greatly above normal, in potassium starvation it departs but slightly if at all from the fully manured value in the early stages of leaf history; and reasons have been presented for the belief that if leaves from the fully manured and potassium-deficient series could be taken at really comparable stages throughout their history, the proportion of amide- to amino-nitrogen would not be very different in the two from the time of leaf expansion until death. Such comparison is rendered difficult owing to the very different duration of life of comparable leaves in the deficient series.

To interpret these ratios of amino- and amide-nitrogen with certainty it is of course necessary to understand the relationship of asparagine to the nitrogen cycle. This is still unknown, but it is usually supposed that its production represents either an essential step in the synthesis of protein or

<sup>1</sup> If this be true, 'storage' or 'reserve' protein would seem to be a singularly inapt description, since one of the main elements so 'stored' cannot be utilized in metabolism, even in a time of very severe shortage.

that it is a by-product of the reactions taking place. According to the latter view it may be conventionally regarded as a temporary and harmless store of excess ammonia until this can be used in protein synthesis, and just possibly as the most convenient substance for translocation of partially elaborated nitrogen to be later re-synthesized in some other region. If any of these statements is correct it is clear that we have the strongest evidence that when phosphorus is deficient nitrogen metabolism beyond the stage of amide production proceeds at a subnormal rate, and that the presence of phosphorus is essential to the building of protein. Hence we find in phosphorus deficiency a subnormal amount of protein, even immediately after expansion of the leaf.

Under potassium starvation, on the other hand, we have a much more nearly normal proportion between amide- and total amino-nitrogen; hence if it is usual for amino acids derived from protein hydrolysis to be deaminated with the formation of asparagine, which in its turn is used for protein re-synthesis, it is clear that there is little evidence of any relative departure from the normal in the rate of production of amide from amino acids compared with the rate of utilization of amide in protein production, such as might be expected if either of these processes were dependent on potassium. It should be pointed out that this difference between the two manurial series occurs in spite of reduced carbohydrate concentration in the potassium-deficient plant, and of a normal or even supranormal concentration under phosphorus deficiency, factors which tend to produce the opposite result.

From the data presented by Burrell (2) it is clear that he too obtained no appreciable departure from the normal in the ratio of amide- to amino-nitrogen under potassium deficiency, but the data of Nightingale *et al.* (25) apparently show a definite increase. In their lower leaf blades the ratio is much higher than in the upper blades, agreeing with the marked rise found with leaf age in all manurings of the present experiments. In the old blades also the difference produced by potassium deficiency is very slight, but the difference in upper blades is considerable. This is probably explained by the shorter life history of deficient leaves, and the upper blades of this series may represent, therefore, on the average, leaves which have passed through a greater proportion of their total history than those from the high-potassium series. In the same way the absolute rate of increase of the ratio with time in the present experiments is much greater under deficiency, although the rate of increase relative to length of life cannot be very different in the two series.

Finally, it may be again pointed out that the close agreement between the distributions of potassium and protein throughout the plant reported by many workers, and, in particular, by Penston (29), by no means necessarily implies any direct connexion between potassium and some phase of nitrogen metabolism. For the distribution of protein is essentially that of proto-

plasm, and if potassium is a prerequisite for any one of the activities or substances constituting living protoplasm, there must inevitably result a close agreement between protein and potassium distributions.

(iv) *General relations in leaves of normal and deficient types.* Beginning with a fully expanded leaf from a normal plant the general picture presented is that hydrolysis of protein proceeds, yielding amino acids and amide. These may be either translocated to the younger parts or largely reconverted into protein *in situ*. In regard to the question of translocation, the total nitrogen figures indicate that in the earlier leaves the supply of nitrogen, after complete expansion of the leaf is not sufficient to counterbalance that leaving the leaf and the content drops with leaf age. Almost the entire supply to the first leaves must be as nitrate, or at least newly assimilated nitrogen, but presumably the successive later leaves may receive degradation products from the ageing leaves below. At the same time the successive leaves are rapidly increasing in size, and the supply of amino acids to one leaf from the much smaller leaves below, together with an ever-diminishing supply of nitrate from the roots, is still insufficient to balance the degradation losses which the leaf itself suffers, with the result that the total nitrogen within it rapidly diminishes in amount. But when maximum leaf-size is passed, and the successive leaves show again a decrease in size, the amino acids reaching them from the larger dying leaves become an important contribution, and we not only find that the last leaf or so at expansion has a higher nitrogen level than those immediately preceding when they were at the same stage, but also that Leaves 8 and 9 (and presumably 10), continue to accumulate nitrogen for some time after expansion. The accumulation of protein resulting from this may possibly represent 'storage' protein, for carbohydrate concentration is at a maximum while leaf growth has ceased.

It is apparent that the life of any one leaf is not continued indefinitely, even if ample nitrogen is presented to it. The first few leaves may die with a higher concentration of nitrogen within them than later leaves ever attain, this being true to a striking extent under nitrogen deficiency. Moreover, in plants amply supplied with nutrients leaves will die with unused nitrate within them, while leaves from nitrogen-starved plants, at a much lower nitrogen level, persist nearly as long as those from fully manured plants. There is definitely some ageing factor other than nitrogen supply, although it is in general true that senescence is accompanied by a loss of this element; protein is hydrolysed more rapidly than it is synthesized, the decomposition products going largely to the younger leaves and the meristems. These conclusions are in general agreement with those of Mothes (22).

At the same time it is probably true that ageing is influenced to some extent by nitrogen and other nutrient relations, and that any loss of nitrogen will accelerate the onset of death. It is known that in many respects leaves

are adjusted to the conditions obtaining during the period of their development, and that under those conditions they are more efficient than those developed in other surroundings. Early leaves develop in the presence of abundant nitrogen, while later leaves start at a much lower level, and in spite of this difference in nitrogen content both types function normally. But when the content is reduced much below the original level, senescence occurs in both types.

If a leaf be separated from the plant it may live considerably longer than if left attached; similarly if the terminal bud be removed from a plant the remaining leaves may survive longer than normally, and even show a pronounced increase in area and thickness (37). Evidently the presence of the meristem reduces the potential life of the leaf produced by its former activity. One important result of isolation from growing regions is that carbohydrates accumulate, and this factor, as has been shown by Mothes and others, is of considerable importance in maintaining protein content within leaves, and is likely, therefore, to influence ageing. The statement of James (15) that 'lack of carbohydrate is presumably a result of leaf degeneration, not a primary cause of it', is therefore not likely to represent the whole truth. Again, removal of the meristem may result in accumulation of nitrogen and other nutrients in existing leaves instead of the more usual steady depletion; and if the general level of nutrients during the growth of a leaf determines the essential conditions for its whole life, so that any large subsequent depletion tends to disintegration, whatever may be the general level of nutrients, this factor may be of importance. It is certainly true that the last few leaves produced by the main axis of barley age much less rapidly than earlier ones, and it is precisely these leaves which have the highest carbohydrate concentration, and in which the nitrogen level, though very low, is maintained for a long time, and even increases after maturity. Very possibly similar considerations hold for other nutrients, including potassium (cf. James, 15). As the meristem grows with the continual production of new primordia, the level of nutrients in the growing regions in general falls, with the inevitable result that the difference in concentration between a mature leaf and the immature parts increases with time. For this reason alone the tendency towards translocation away from the leaf probably increases progressively with time, quite apart from any conditions internal to the leaf itself; herein may lie the cause of the reduction in potential length of life ascribable to the presence of the meristem.

Protein nitrogen, expressed in terms of dry weight, drops markedly with leaf age, though in later leaves it accumulates for some time after expansion. As a percentage of total nitrogen it changes only slightly with age in fully manured plants, and at death there is still much protein present (cf. Chibnall, 5). Usually protein accounts for 80 to 85 per cent. of the total nitrogen within the leaf, but the relative amount tends to increase in

later ones, the increase being mainly due to a falling nitrate content in successive leaves. Amino-nitrogen is also a fairly constant proportion of the total both between the various leaves and within any one leaf with age, though there may be in general a slight tendency to rise with age while protein tends to fall. In leaves of these plants, therefore, the general relationship between protein and amino-nitrogen simulates that of a chemical equilibrium, but under deficiency this relationship is disturbed. Chibnall (3) also found in leaves of the runner bean throughout life history a nearly constant proportion of protein (69 to 77 per cent. total nitrogen) and that monoamino-nitrogen varied directly with protein.

In the case of leaves from a potassium-deficient plant, owing probably to protoplasmic breakdown primarily unconnected with the nitrogen cycle the rate of protein hydrolysis is quicker, leading to a high concentration of amino- and amide-nitrogen. Very early death supervenes. The higher soluble nitrogen concentration within them will involve a greater rate of translocation from the leaves, leading to a rapid loss of nitrogen towards the active meristem. With an abundant supply of organic nitrogen, nitrate is but little used, and accumulates in the plant. The meristem having a supranormal supply of nitrogenous material, and being also moderately well supplied with potassium is enabled to synthesize protein without excessive hydrolysis, hence the leaves begin their history with at least a normal protein content; even so, the protoplasm at this stage does not function normally, as is evidenced by a greatly increased respiration rate, and possibly by the nearly complete cessation of photosynthesis (11, 34).

Under phosphorus deficiency the products of hydrolysis within the leaf are not readily re-synthesized and accumulation of amino acids and especially of amide occurs. Protein content, therefore, drops rapidly, and death is early. But in this case also synthesis at the meristem cannot proceed at the normal rate in spite of a relatively higher concentration of phosphorus there (8, 21), and the youngest analysed samples of leaves show subnormal protein contents. High concentrations of amino acids and asparagine may thus occur everywhere (cf. 21). This factor, together with the relative inactivity of the meristem, will tend to a less rapid depletion of the nitrogen of the leaf than in the case of potassium starvation, and this is in general found. The low rate of meristematic synthesis is reflected in reduction of leaf-size and rate of leaf and tiller production, features similar to those found under nitrogen starvation, but in contrast to those of potassium shortage in the present experiments.

Although the above interpretation of the results appears to the authors to be the most rational in the present state of knowledge, other possibilities must not be ignored. Thus it has been shown that in all manurings amide, expressed as a percentage of total nitrogen, increases with leaf age, and particularly so towards the end of the leaf's history in the fully manured

and nitrogen-starved series. The same is generally true of total amino-nitrogen, but this is mainly due to the inclusion of the amino group of asparagine; for if amide- be subtracted from total amino-nitrogen, the amino acids remaining show a marked tendency to fall again towards the end of the life history. Even on a percentage dry-weight basis the same distinction appears to hold. There are at least three possible explanations of this: (1) the amino acids produced by hydrolysis tend to be rapidly converted into asparagine before removal or re-synthesis; (2) as proteins are hydrolysed to asparagine and amino acids the latter are translocated rapidly away while the asparagine is only slowly removed; or (3) the later proteins to be hydrolysed yield directly higher amounts of asparagine than those first attacked.

In the preceding discussion the first of these explanations has been emphasized, although some observations by Chibnall (5) lend a certain amount of support to the third. He found that the proteins in dead runner bean leaves are slightly different from those of living leaves, as on hydrolysis they yield greater amounts of amide-nitrogen. It seems unlikely that some such difference in kind will wholly account for the extremely abnormal ratio of amide- to amino-nitrogen in the present phosphorus-deficient series, but some differences in the nature of protein with manurial treatment are very possibly present, and the general effect of potassium or phosphorus starvation on the nitrogen cycle may be partly due to an unusual route, and not entirely to altered rates at certain points of the cycle. Indeed, it appears almost inevitable that such differences must exist under phosphorus deficiency, when the direct association of phosphorus with some proteins and not with others is considered. The present experiments cannot elucidate the matter.

(v) *Nitrate and reductase activity.* In conclusion, reference may be made to the nitrate figures and their bearing on the question of reductase activity in plants. It has been shown that under potassium deficiency the later leaves accumulate nitrate as they age, and die with it still unused; the same is true, though to a less extent, of phosphorus deficiency. Similar observations have been made by several other workers, and Eckerson has examined the reductase activity of many manurial and other types (7, 8, 9). She came to the conclusion that potassium and phosphorus are essential to its activity, and plants deficient in these elements are unable to reduce nitrate. It is held that the same is true to a less extent of plants deficient in calcium or sulphur, and of those grown under reduced light or in a short day. She therefore attributes the early high carbohydrate phase of Nightingale's potassium-deficient plants, and the similar early phases of phosphorus deficiency, to a break-down of protein synthesis at the first stage of the nitrogen cycle. But it is difficult to believe that plants deprived of potassium, which at all times contain normal or supranormal percentages

of organic nitrogen and normal amounts of protein, can be suffering in any way from a lack of nitrogen. The first detectable effect of potassium deficiency on the nitrogen cycle is an increased proportion of amino acids, and unless these cannot be utilized in the re-synthesis of protein the plant has available for synthesis an unusually high supply of partially elaborated nitrogen. Further reduction of nitrate until this type of nitrogen is decreased in amount is therefore unnecessary, and may be detrimental. The same is true of phosphorus deficiency ; for although protein, and even total nitrogen, may be reduced, yet again the amount of partially elaborated nitrogen is excessively high. It would appear that almost all the circumstances leading to a diminution of reductase activity, with the probable exception of calcium deficiency (26), lead also to an excess of the products of protein-hydrolysis ; while under nitrogen deficiency, in which amino acids are reduced in amount, reductase activity is increased.

It is probable that the inverse correlation between amino acid concentration and reductase activity is significant, and that there exists in plants a mechanism for the reduction of nitrate which is to some extent dependent on the amount of amino acids present. If this is the case the formation of organic nitrogenous compounds will not proceed indefinitely but only in so far as these are utilized in further growth ; moreover, as the level of nitrate within the plant rises, the rate of entry will decrease. Therefore a further consequence of such a reduction mechanism is that total nitrogen content will not rise to an inordinately high level, and that there is a definite limit to the so-called 'luxury consumption' of nitrate

#### CONCLUSIONS AND SUMMARY.

1. An experiment is described in which barley was grown in sand culture under four nutrient treatments, i.e. complete nutrients and deficiency of nitrogen, of phosphorus, and of potassium. On eight selected dates during the vegetative period samples of all the living leaves on the main axis were taken. The successive individual leaves were grouped separately so that changes with time in the composition of each leaf could be observed. Leaf samples were dried immediately, and subsequently analysed for certain nitrogen fractions. The analytical results are presented in terms of percentage dry weight and percentage total nitrogen.

2. Differences in composition of the successive leaves at the time of their emergence were found, together with differences in the changes occurring as the leaves age. In general, total nitrogen and most of the estimated fractions rose to a maximum about the period of Leaves 2-4, and thereafter declined to a minimum at Leaf 8 or 9, the last leaves again showing a rise. Nitrogen content fell continuously with leaf age in the earlier leaves, but in later ones rose for some time after expansion. These



changes are discussed in relation to the amount of nitrogen available at different periods, two main sources being recognized, namely, newly absorbed nitrogen and nitrogen liberated in ageing leaves by the hydrolysis of protein.

3. Differences in nitrogen level induced by deficiency of that element are very marked, but there is little indication of departure from the usual protein cycle, and the observed fractions bear much the same relations to one another as in high-nitrogen plants.

4. Under phosphorus deficiency large differences are found. Protein is reduced even in early stages of the leaf history, and rapidly declines with the age of the leaf. Although there is some accumulation of nitrate in later leaves and pronounced accumulation of amino-nitrogen, the most marked characteristic of phosphorus deficiency is a greatly increased amide concentration. Clearly there is a check in protein synthesis beyond the stage of the production of asparagine. The inability to synthesize adequate amounts of protein is held to be the primary cause of the similarity between many of the symptoms of phosphorus and of nitrogen deficiency, leading in both cases to low meristematic activity as is indicated by reduction in tillering rate, leaf production rate, and individual leaf size.

5. The main effects of potassium deficiency on the various fractions are (1) a very rapid disappearance of protein with advancing leaf age although at the time of emergence these leaves have a normal protein content; (2) a marked increase in amino- and amide-nitrogen, though the relation between these two fractions appears to be normal; and (3) accumulation of nitrate in later leaves. The very different types of plants obtainable under varied conditions of potassium deficiency are stressed. The hypothesis that potassium plays an essential part in protein synthesis is examined, and an alternative view that appears to be more in accord with the known facts is presented. It is held that potassium is not primarily connected with protein synthesis, but is in some manner essential to the maintenance of the protoplasmic complex, and that in its absence protoplasm rapidly breaks down, leading to very early death of leaves. In consequence protein is rapidly hydrolysed, and accumulation of simpler nitrogenous substances occurs throughout the plant.

6. The relation between nitrogen supply and ageing of the leaf is discussed.

7. The observed accumulations of nitrate under phosphorus and potassium deficiency are examined in relation to views expressed in the literature regarding the influence of these elements on reductase activity. A possible relation between reductase activity and amino acid concentration is indicated.

The above work was performed at Rothamsted, and the authors' thanks are due to Sir E. J. Russell for granting the necessary facilities.

It is a pleasure also to record our indebtedness throughout the work to Professor V. H. Blackman and Dr. F. G. Gregory, while for assistance in the early stages grateful acknowledgement is made to Professor A. C. Chibnall and to Dr. L. R. Bishop.

# ADDENDUM.

While this paper was passing through the press the authors have seen the account of Mothes' work relating the activity of proteolytic enzymes to oxidation. It is realized that this important work may have considerable bearing on some of the questions raised in the present paper; any discussion from this point of view is, however, withheld for the present.

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# The Interaction of Factors in the Growth of Lemna.

## VIII. The Effect of Nitrogen on Growth and Multiplication.

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With eight Figures in the Text.

### EXPERIMENTAL PROCEDURE.

THE same technique as that previously described (6) was used for a study of the effect of nitrogen concentration at 25° C. and under continuous illumination of 450 foot-candles, except that 150 c.c. pyrex beakers were substituted for those of silica. Colonies were grown in nitrogen concentrations<sup>1</sup> of 1, 5, 10, 25, and 100 mg. per litre. A colony grown without added nitrogen ceased to multiply after six days. All colonies were transferred to fresh nutrient solution daily.

*Fronde number.* The growth data are summarized in Table I. The area and dry weight values are the means of samples taken after the fourteenth day for all treatments save that without added nitrogen.

TABLE I.

Nitrogen (mg. per litre).	Relative frond number increase.	Average frond dry weight (mg.).	Average frond area (sq. cm. with S. E.).	Dry weight per unit area (mg. per sq. cm.).	Net assimila- tion rate (mg. CO <sub>2</sub> per sq. dm. per hour).
0	0.101	0.127	0.0278	4.57	5.92
1	0.231	0.112	0.0368 ± 0.0010	3.07	5.43
5	0.302	0.120	0.0542 ± 0.0007	2.21	4.54
10	0.299	0.104	0.0500 ± 0.0010	2.16	4.15
25	0.305	0.106	0.0467 ± 0.0006	2.30	4.69
100	0.299	0.100	0.0443 ± 0.0012	2.25	4.60

No further increase in rate of frond multiplication occurs on raising the nitrogen concentration above 5 mg. per litre. The relative rate of increase in a solution containing 1 mg. nitrogen per litre remained identical with those at higher concentrations for eight days. Subsequently the rate fell away, while simultaneously a rising trend in the curve of dry weight per

<sup>1</sup> CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub> 0.101 gm.; Mg.SO<sub>4</sub> 0.255 gm.; K<sub>2</sub>SO<sub>4</sub> 0.668 gm.; Fe<sub>2</sub>Cl<sub>6</sub> 0.002 gm.; dist. water 1 litre; Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O 0.581 gm.; Mg(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O 0.382 gm.; stock solution of mixture of Ca and Mg nitrates diluted for lower nitrogen concentrations.

unit area appeared. With no added nitrogen the relative rate of increase fell so rapidly that increase in frond number ceased six days after the omission of nitrogen from the solution. With this treatment a linear relationship is obtained by plotting the logarithms of frond number against the logarithms of time and the following equation is fitted by the method of least squares:

$$(1) \quad \frac{1}{n} \frac{dn}{dt} = \frac{0.310}{t} \quad \log n = 0.310 \log t + 2.386.$$

The rate of growth during starvation may also be considered as a decrement from the rate prior to starvation, the change of rate following a logarithmic decrement law. On the basis of this assumption the following equation is fitted, 0.245 being the rate at the beginning of the starvation period which falls during starvation at a constant rate of 32 per cent. per day:

$$(2) \quad \frac{1}{n} \frac{dn}{dt} = 0.245 e^{-0.319 t} \quad \log \frac{n}{n_0} = \frac{0.245}{0.319} (1 - e^{-0.319 t}).$$

The two constants of this equation are obtained by the method of least squares, and the frond numbers calculated from the mean number during the starvation period are shown in the fourth column of Table II. The frond numbers calculated from equation (1) are shown in column three.

The observed and calculated values are shown in Table II. For the remainder of the experiment this colony was grown in a solution with 5 mg. nitrogen per litre in order to study the mode of recovery from nitrogen starvation.

TABLE II.

*Frond Numbers of a Colony with no Added Nitrogen.*

Day.	Obs.	Calc. (1).	Calc. (2).
0	209	—	208
1	276	—	257
2	292	301	300
3	356	342	335
4	378	374	364
5	407	401	386
6	424	424	403

*Average frond area.* Fig. 1 shows the relation between average frond area and nitrogen concentration. Both low and high nitrogen concentrations lead to decrease in frond area, the optimal concentration being 5 mg. per litre. That the fall in average frond area of colonies grown in high nitrogen concentrations is real is shown by the following considerations:

(1) The mean difference in average frond area between the colonies grown with 5 and 100 mg. per litre exceeds by over seven times the standard error of the mean difference (samples taken during the last four days of the experiment).

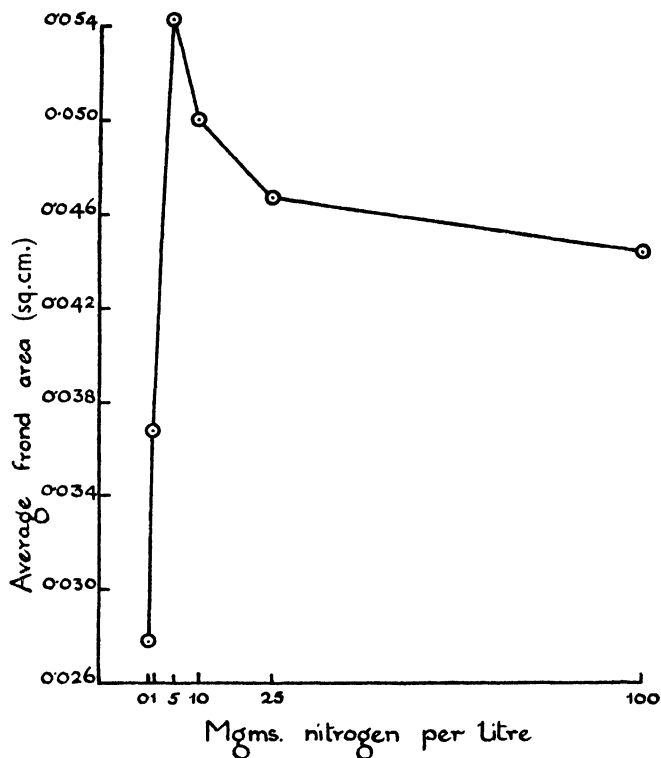


FIG. 1. Average frond area plotted against nitrogen concentration.

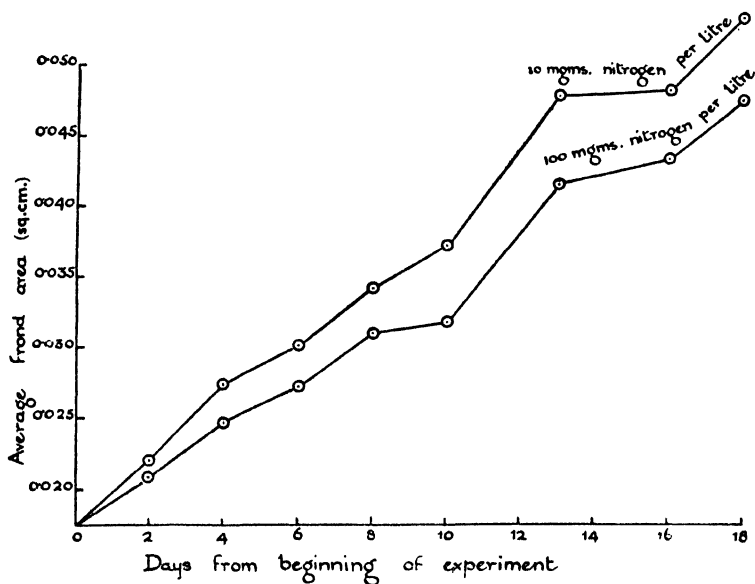


FIG. 2. Average frond area of colonies growing in solutions with 10 and 100 mg. nitrogen per litre.

(2) The average frond areas of the colonies in solutions with 10 and 100 gm. per litre show a steady and progressive divergence as the experiment proceeds, as shown in Fig. 2.

TABLE III.

*Length of Root.*

Nitrogen. per litre (mg.).	Mean length (cm.) of root (with S. E.).
0	3.0 ± 0.2
1	4.9 ± 0.2
5	3.9 ± 0.3
10	3.6 ± 0.2
25	3.7 ± 0.2
100	2.9 ± 0.1

Table III shows the relation between nitrogen supply and length of root. Maximal length of root occurs in a concentration of 1 mg. nitrogen per litre. Increase in nitrogen supply from 1 to 100 mg. per litre is associated with decreasing length of root and decrease in nitrogen supply from 1 to 0 mg. added nitrogen is associated with a marked reduction in root-length.

The fronds of the colony with 5 mg. nitrogen per litre were deep green, denoting high chlorophyll content. Either increase in nitrogen supply to 100 mg. per litre or decrease below 5 mg. per litre leads to reduction in chlorophyll content. Increase in nitrogen supply above 5 mg. per litre does not affect the multiplication rate but reduces the frond area, chlorophyll content, and length of root, and a concentration of 100 mg. per litre must be considered supra-optimal under the experimental conditions. The nitrogen content of Clark's solution (1) of 112 mg. per litre appears to be too high for maximal growth.

*Dry weight.* The dry weight per unit area of colonies with 5 mg. nitrogen per litre, 1 mg. nitrogen per litre, no added nitrogen, and also during recovery from nitrogen starvation is shown in Table IV and Fig. 3. The high dry weight per unit area of nitrogen-starved fronds was shown, by decolorizing fronds and staining with iodine, to be associated with high starch content.

The relation between average frond area and dry weight per unit area at different nitrogen concentrations is inverse. The values given in Table I correspond with a correlation coefficient of  $-0.887$  ( $P=0.02$ ). It may readily be seen that variation in nitrogen supply has only slight effect upon average frond dry weight. A similar reduction in average frond area associated with increased weight per unit area has been demonstrated with potassium starvation (6).

*Recovery from nitrogen starvation.* Fig. 4 shows that at the end of a period of three days, roughly corresponding to a single generation, the

relative rate of frond number increase of a colony, previously nitrogen-starved, does not differ significantly from that of a colony grown in the same nitrogen concentration since the beginning of the experiment.

Fig. 5 shows the average frond area and dry weight during starvation

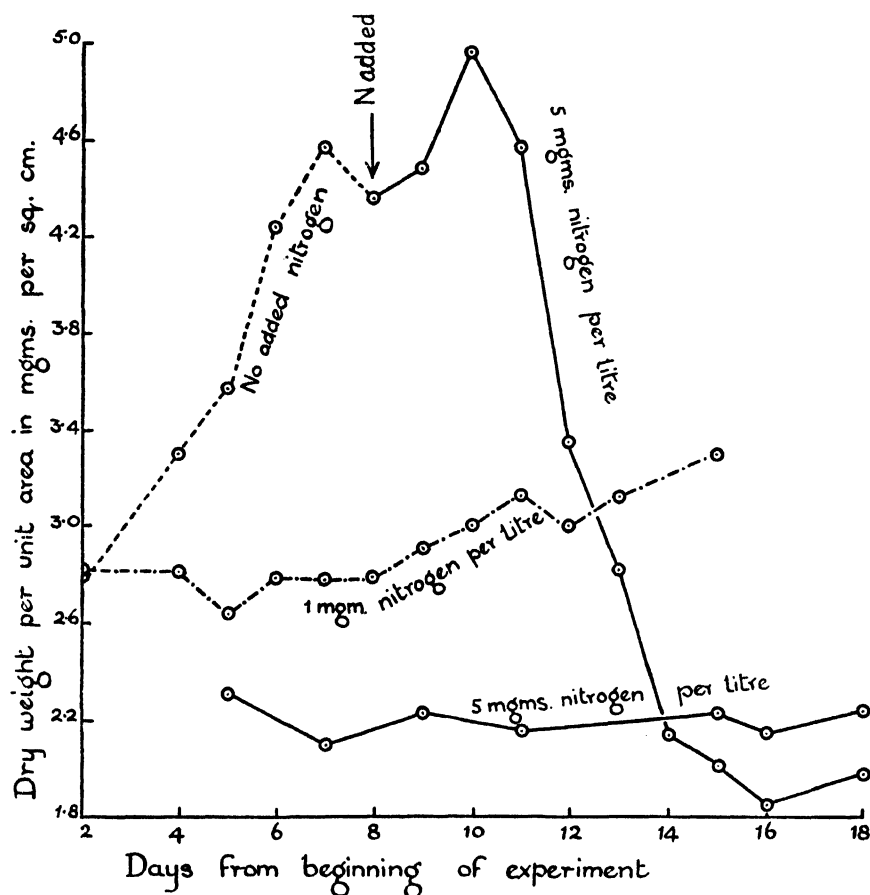


FIG. 3. Dry weight per unit area of colonies growing in solutions with 1 and 5 mg. nitrogen per litre and of a colony with no added nitrogen, transferred after 6 days of starvation (8th day of the experiment) to a solution with 5 mg. nitrogen per litre.

and recovery, as percentage changes from the corresponding colony grown continuously with 5 mg. nitrogen per litre. During the period of starvation the frond dry weight of the starved colony shows a progressive gain on that of the colony with optimal concentration of 5 mg. nitrogen per litre. On addition of nitrogen to the solution the values of the starved colony fall rapidly and reach temporarily a level lower than that of the control colony.

Two points characterize recovery from nitrogen starvation and contrast with recovery from potassium starvation.



TABLE IV.

*Dry Weight per Unit Area (mg. per sq. cm.) 450-ft. Candles.*

Day.	0 mg. nitrogen per litre.		5 mg. per litre after 6 days' starvation.		1 mg. nitrogen per litre.		5 mg. nitrogen per litre.	
2	2.79	2.79			2.75	2.82		
	2.79				2.89			
3								
4	3.28	3.30			2.82	2.81		
	3.33				2.81			
5	3.86	3.57			2.64	2.64	2.30	2.30
	3.28							
6	4.17	4.24			2.70	2.79		
	4.32				2.89			
7	4.67	4.57			2.74	2.78	2.06	2.09
	4.47				2.83		2.12	
8	4.23	4.36			2.67	2.79		
	4.50				2.71			
9			4.58	4.47	2.87	2.91	2.31	2.23
			4.37		2.95		2.16	
10			5.04	4.96	2.99	3.00		
			4.89		3.01			
11			4.76	4.56	3.11	3.13	2.12	2.16
			4.36		3.15		2.21	
12			3.16	3.35	3.00	2.99		
			3.54		2.98			
13			2.89	2.82	3.09	3.12		
			2.76		3.16			
14			2.14	2.14				
15			2.10	2.02	3.19	3.30	2.16	2.23
			2.95		3.41		2.30	
16			1.87	1.85			2.18	2.15
			1.84				2.12	
17								
18			1.86	1.98			2.36	2.24
			2.11				2.13	

(1) The dry weight per unit area, already at a high value during starvation, rises still higher immediately nitrogen is supplied and does not fall until three days later (Fig. 3), *after* the relative rate of increase in frond number has risen to a value compatible with that of a colony grown continuously in a solution with 5 mg. nitrogen per litre (Fig. 4).

(2) There is a lag period, extending over at least two generations, in recovery of average frond area, lower values being found during recovery than during starvation (Fig. 5). This must be attributed to the rapidity of fall in rate of multiplication with nitrogen starvation, so that increase in

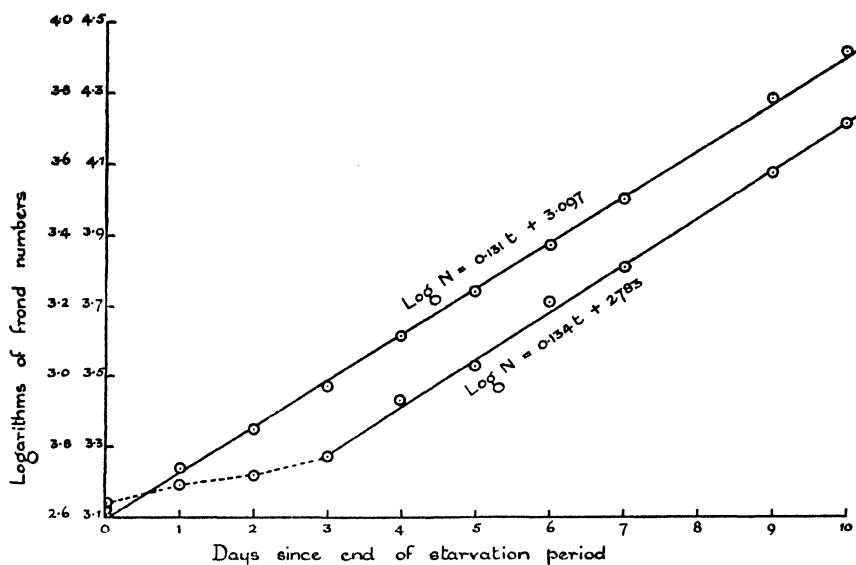


FIG. 4. Logarithms of frond number plotted against time of (a) a colony, previously nitrogen-starved, after transference to a solution with 5 mg. nitrogen per litre, and (b) a colony grown continuously with 5 mg. nitrogen per litre.

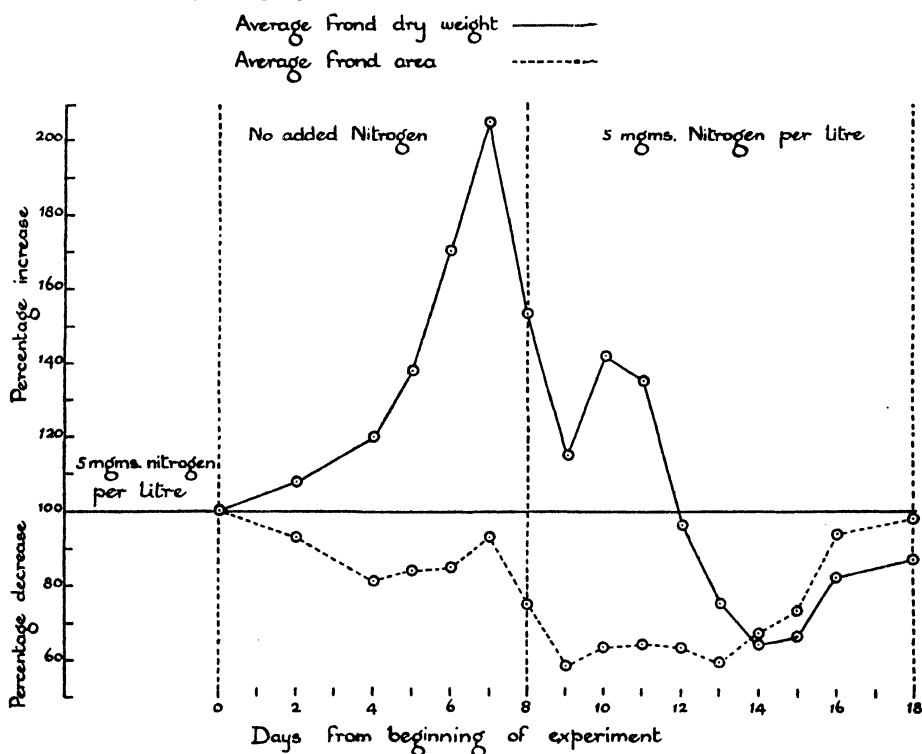


FIG. 5. Percentage relation, in average frond area and dry weight, of a colony transferred, after a period of nitrogen starvation, to a solution with 5 mg. nitrogen per litre, to the area and dry weight of a colony growing continuously in a solution with 5 mg. nitrogen per litre, which is represented by a straight line.

frond number soon ceases. In consequence the relative number of small fronds formed under conditions of starvation is low, and the *average* frond area is not markedly reduced. On transference to a solution with full nitrogen supply the new fronds formed, which are at first small, reduce the

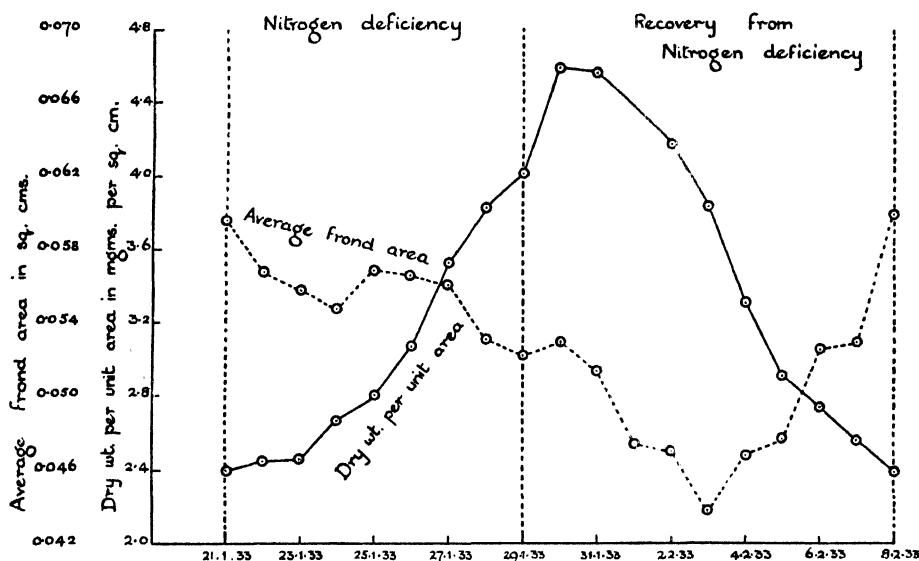


FIG. 6. Average frond area and dry weight per unit area during an 8-day period of nitrogen deficiency followed by transference of the colony to a solution with full nitrogen supply.

average frond area to a lower level than during starvation. With potassium starvation increase in frond number continues, under corresponding conditions, for a period about three times as long as with nitrogen starvation. The new fronds formed are increasingly small in size, so that the preponderance of new fronds tends to reduce the *average* area per frond. On transference to a solution with full potassium supply the new fronds formed are larger and tend therefore to raise rather than reduce the average frond area of the colony.

A later experiment afforded opportunity to confirm these observations; recovery from nitrogen starvation was studied under a light intensity of 900-ft. candles. Table V and Fig. 6 give the average frond area and dry weight per unit area during starvation and recovery. The rise in dry weight per unit area immediately on supplying nitrogen and lag in recovery of average frond area confirm the previous experiment at 450-ft. candles.

*Respiration rate.* The writer is indebted to Mr. W. G. Templeman for carrying out respiration determinations, by a modification of a Warburg manometric method, on samples similar to those used for area and dry weight determinations. The results have been calculated by the writer for each of nine hours from the commencement of the determinations and are given in

TABLE V.

Date.	Average frond area (sq. cm.).		Dry weight per unit area (mg. per sq. cm.).	
	900-ft. candles.			
	0.1 mg. nitrogen per litre.	Full nitrogen supply after 8 days' deficiency.	0.1 mg. nitrogen per litre.	Full nitrogen supply after 8 days' deficiency.
21. 1. 33	0.0592 0.0600	0.0596	2.38 2.40	2.39
22. 1. 33	0.0559 0.0577	0.0568	2.50 2.39	2.45
23. 1. 33	0.0561 0.0554	0.0558	2.42 2.49	2.46
24. 1. 33	0.0556 0.0539	0.0548	2.66 2.65	2.66
25. 1. 33	0.0557 0.0581	0.0569	2.84 2.76	2.80
26. 1. 33	0.0564 0.0568	0.0566	3.11 3.05	3.08
27. 1. 33	0.0550 0.0569	0.0560	3.46 3.60	3.53
28. 1. 33	0.0531 0.0531	0.0531	3.77 3.87	3.82
29. 1. 33	0.0536 0.0507	0.0522	4.07 3.95	4.01
30. 1. 33		0.0529		4.59
31. 1. 33		0.0514		4.56
1. 2. 33		0.0474		—
2. 2. 33		0.0470		4.17
3. 2. 33		0.0438		3.83
4. 2. 33		0.0468		3.31
5. 2. 33		0.0477		2.91
6. 2. 33		0.0526		2.74
7. 2. 33		0.0530 } 0.0529		2.66 } 2.56
		0.0528 }		2.46 }
8. 2. 33		0.0599		2.39

TABLE VI.

*Respiration Rate (mg. CO<sub>2</sub> per sq. dm. per hour) of a Colony Previously Nitrogen-deficient and Receiving from 29.1.33 Onwards Full Nitrogen Supply.*

(The figures in the first column show the successive hours of darkness.)

	28. 1. 33	30. 1. 33	31. 1. 33	1. 2. 33	2. 2. 33	3. 2. 33	4. 2. 33	6. 2. 33
1	1.30	0.82	1.72	3.24	4.62	4.00	3.15	1.18
2	1.38	1.14	1.92	5.59	6.18	3.09	1.67	1.45
3	1.47	1.36	2.02	5.19	3.05	4.01	1.76	1.70
4	1.53	1.23	1.91	4.50	2.16	2.10	1.96	1.76
5	1.66	1.11	1.87	1.94	1.99	1.96	1.90	1.91
6	1.53	1.00	1.69	1.70	1.89	2.24	1.77	1.85
7	1.34	0.98	1.52	1.65	1.79	1.41	1.67	1.71
8	1.29	1.10	1.35	1.52	1.70	1.43	1.65	1.69
9	1.19	1.09			1.61	1.33		1.61

Table VI and Figs. 7 and 8. Table VI suggests an explanation for the temporary rise in dry weight per unit area on supplying nitrogen to a starved colony (Figs. 3 and 6), for the first set of respiration determinations (30.1.33) after transference to a solution with full nitrogen supply is lower than that (28.1.33) at the end of the starvation period.

From the respiration determinations two conclusions appear to be established.

(1) The respiration of a nitrogen-starved colony, illustrated by a comparison of the results of 28.1.33 (during starvation) with those of 6.2.33 (after recovery), is lower than that of a colony with full nitrogen supply.

(2) The respiration rate rises temporarily during the transition period between nitrogen starvation and final recovery to a high level, in this experiment of the order of five times its previous and subsequent values.

Comparison of the curves of respiration rate with Fig. 6 shows that the fall in dry weight per unit area follows the rise in respiration rate and is presumably associated with the hydrolysis of surplus starch, stored up during the starvation period. Variation in respiration in excess or deficiency of the rate found at the end of the recovery period has been converted into dry weight gain or loss on the assumption that starch is the material respired. Taking the means of the respiration rates during the second and third hours of measurement as characteristic, then the total calculated loss of weight by respiration during the transition period between starvation and final recovery corresponds to a fall in dry weight per unit area of 1.4 mg. per sq. cm., whereas the total fall actually recorded is 1.85 mg. per sq. cm. Thus 75 per cent. of the fall in dry weight per unit area is accounted for.

The high respiration rate during recovery corresponds with a temporary surplus of respiratory material following rapid hydrolysis of starch stored during starvation. This was confirmed by microchemical tests for starch in the 450-ft. candle experiment. The starch content fell throughout the recovery period until on the eighth day of recovery no trace of starch could be found, this being the only occasion throughout the whole series of experiments that fronds were observed to be entirely free from starch. This supported the view that the high starch content and low respiration rate during starvation might be associated with a low rate of starch hydrolysis. By the method previously described (6) the amylolytic activity was measured by the time taken by samples of equal dry weight to hydrolyse a given starch solution. Under 900-ft. candles this was found to be about 21 hours during nitrogen starvation and 2 hours with full nitrogen supply.

Rough estimates of the protein content of the fronds were made by decolorizing in alcohol and staining with Millon's reagent. As was to be expected, the pale yellow nitrogen-starved fronds had a low protein content.

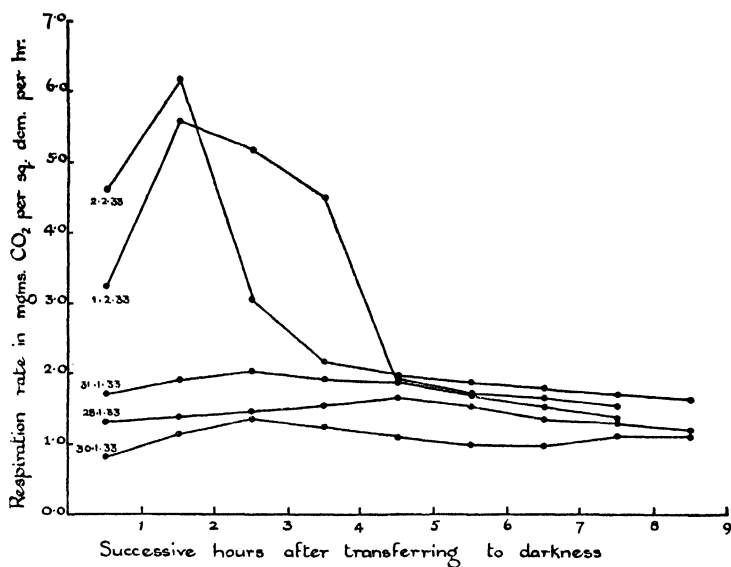


FIG. 7. Rates of respiration, for each of 9 hours after transference to darkness, of a colony during the transition period between nitrogen starvation and recovery.

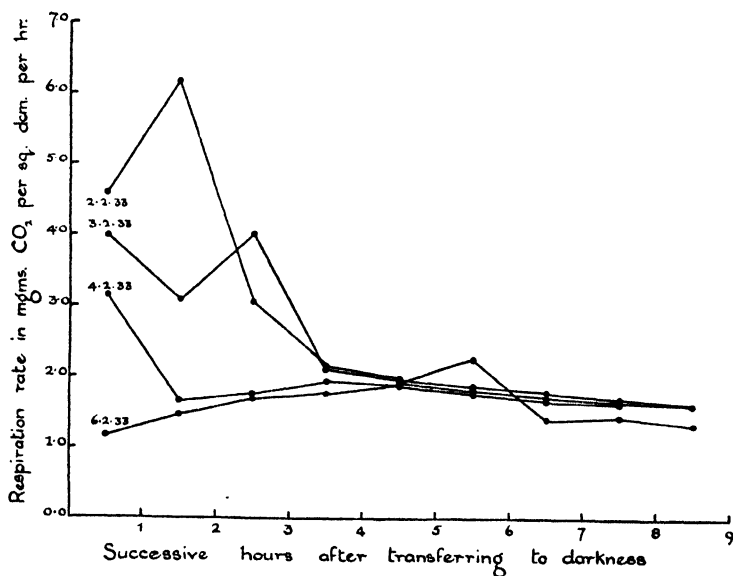


FIG. 8. Rates of respiration, for each of 9 hours after transference to darkness, of a colony during the transition period between nitrogen starvation and recovery.

## DISCUSSION.

(A) *The effects of variation in nitrogen supply.* The effects of variation in nitrogen supply may be summarized as follows: (1) A colony exposed to *severe* nitrogen starvation is characterized by falling multiplication rate, low frond area, low respiration rate, low protein content, low chlorophyll content and reduced amylolytic activity, high net assimilation rate, high dry weight per unit area and high starch content, and a *short* root. (2) A *moderately low* nitrogen supply is associated at first with a steady, but afterwards by a falling, multiplication rate, low frond area, high net assimilation rate and high dry weight per unit area, and a *long* root. (3) Increase in nitrogen supply from an optimal concentration of 5 mg. per litre to 100 mg. per litre does not affect multiplication rate, net assimilation rate, or dry weight per unit area, but leads to low frond area, reduction in chlorophyll content, and a short root.

The outstanding feature of severe nitrogen starvation is the high carbohydrate content of the plants. Carbohydrate accumulation in nitrogen-starved plants is not a simple process, but is affected by at least three sets of changes.

(1) *Change in rate of multiplication.* Provided that the real assimilation rate is unaffected, a falling multiplication rate must be associated with carbohydrate accumulation owing to reduced carbohydrate consumption in the building of new tissue. Fig. 5 demonstrates the progressive increase in dry weight which accompanies the falling multiplication rate (Table II) of the colony grown with no added nitrogen. The dry weight per unit area of the colony in a concentration of 1 mg. nitrogen per litre remains steady so long as an exponential rate of multiplication is maintained. The appearance of a rising trend in the curve of dry weight per unit area (Fig. 3, Day 9) synchronizes with the onset of a falling multiplication rate. These considerations suggest that the most important process leading to carbohydrate accumulation is reduction in carbohydrate consumption due to a falling multiplication rate.

(2) *Change in rate of respiration.* The high net assimilation rate associated with low nitrogen supply (Table I) cannot arise merely from a falling multiplication rate since the method of calculation takes this value into account. The decrease in respiration rate (Table VI) is roughly of an order that could account for the increase found in net assimilation rate, and it is probable that the value of net assimilation is determined by respiration rate, the real assimilation being unaffected by nitrogen supply.

(3) *Change in rate of enzymic hydrolysis of starch.* The enzymic hydrolysis of starch has been found to be retarded by low nitrogen supply (p. 412).

Length of root is affected not only by sub-optimal nitrogen concentra-

tions but also by variation in high levels of nitrogen supply (Table III), which do not affect multiplication rate or assimilation rate (Table I). A direct relation between nitrogen supply and length of root cannot be traced (Table III), though it seems probable that a very low level of nitrogen supply would be associated with a short root since continuous reduction in the nitrogen supply must eventually limit directly all meristematic activity. At higher concentrations the level of nitrogen supply is presumably effecting changes in the concentration of substances, probably sugars, that are of prime importance in determining root-length.

The inference that carbohydrate changes are regulated by variation in the salt supply has been reached previously (6) in the case of potassium starvation. There are striking similarities in dry weight changes during starvation and recovery that suggest partial association between the phenomena of nitrogen and potassium starvation.

(B) *The metabolism of nitrogen-starved and potassium-starved colonies.* Spoehr and Magee (5) have demonstrated an association between respiration rate and amino-acid content. Sherman and Walker (4) have demonstrated an association between amino-acid concentration and amylolytic activity, either because the enzyme is itself derived from protein (3) or because of its inability to function without a protein substrate (2). In nitrogen-starved *Lemna* low respiration rate and low amylolytic activity are linked with low protein content. The association of potassium starvation with low amylolytic activity and high starch content has previously been recorded (6). The protein content of *Lemna* colonies grown in concentrations of 200, 2, 0.125, and 0 mg. potassium per litre was found to give values of 9, 8, 6, and 4 respectively on an arbitrary scale from 10 (high protein) to 0 (no protein) according to the depth of colour given by staining with Millon's reagent. The relative amylolytic activities, as previously recorded (6), were 7.3, 5.9, 3.4, and 1.0 on a weight basis.

Severe potassium and nitrogen starvation both lead in *Lemna* to less amylolytic activity together with the accumulation of surplus starch. On transference of the nitrogen-starved colony to a solution with full nitrogen supply the surplus starch is dissipated rapidly in association with high respiration rate. With potassium starvation direct respiration determinations are not available, but the increase of a potassium-starved colony, two days after transference to a solution with full potassium supply (6), by 94 per cent. in area and *only 16 per cent. in dry weight* is very difficult to account for save by the view that the rate of respiration must have risen appreciably. It is reasonable to infer that the changes in protein content, shown by microchemical tests to be common to both types of starvation, are responsible, by controlling the amylolytic activity, for similarity in dry weight changes of *Lemna* colonies during nitrogen and potassium starvation and recovery.



## SUMMARY.

1. A study has been made of the growth and multiplication of colonies of *Lemna minor* in a series of nitrogen concentrations ranging from no added nitrogen to 100 mg. per litre.

2. Under a continuous light intensity of 450 ft.-candles at 25° C. the optimal nitrogen concentration is of the order of 5 mg. per litre. The effects of variation in nitrogen supply are as follows:

Severe nitrogen starvation. (No added nitrogen.)	Moderately low nitrogen supply. (1 mg. per litre.)	High nitrogen supply. (100 mg. per litre.)	
Falling multiplication rate.	Steady, followed by a falling multiplication rate.	Multiplication rate.	Un-affected.
Low frond area.	Low frond area.	Net assimilation rate.	
High dry weight per unit area.	High dry weight per unit area.	Dry weight per unit area.	
High starch content.	High net assimilation rate.	Low frond area.	
Low protein content.	Long root.	Low chlorophyll content.	
Low chlorophyll content.		Short root.	
Low respiration rate.			
Low amyolytic activity.			
Short root.			

3. The rate of increase in frond number is exponential in concentrations ranging from 5 to 100 mg. nitrogen per litre. The equation of growth may be written  $n_1 = n_0 e^{rt}$  and the differential equation  $\frac{1}{n} \frac{dn}{dt} = r$ .

With no added nitrogen the rate of increase in frond number falls with time. The equation of growth may be written  $n_1 = n_0 t^r$  and the differential equation  $\frac{1}{n} \frac{dn}{dt} = \frac{r}{t}$ . The rate of growth during nitrogen starvation may also be considered as a decrement from the rate prior to starvation, the change of rate during starvation, following a logarithmic decrement law.

The relative growth rate may be represented by the equation  $\frac{1}{n} \frac{dn}{dt} = r e^{-kt}$

and the frond number at any time  $t$  by the equation  $\log \frac{n}{n_0} = \frac{r}{k} (1 - e^{-kt})$ .

4. The average frond area and dry weight per unit area vary inversely over the range of concentrations studied.

5. A study has been made of recovery from nitrogen starvation. During the transition period between starvation and final recovery the high dry weight per unit area, characteristic of starvation, falls rapidly in association with disappearance of starch and high respiration rate. The respiration rate rises temporarily to a level of the order of five times that during starvation. The total loss of dry weight per unit area corresponds roughly with the magnitude of the increase in respiration rate.

6. The effect of variation in nitrogen supply is discussed. Carbohydrate accumulation in nitrogen-starved plants is considered to be due

mainly to a falling multiplication rate which is associated with reduced carbohydrate consumption in growth. Carbohydrate accumulation is accentuated by decreased respiration leading to high net assimilation rate. Reduction in level of nitrogen supply is also associated with low amylolytic activity.

7. The similarity in dry weight changes of nitrogen-starved and potassium-starved colonies is pointed out and their metabolism briefly discussed.

Grateful acknowledgements are due to Professor V. H. Blackman for the provision of facilities for carrying out the work and to Dr. F. G. Gregory for assistance in the preparation of the paper.

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## NOTES.

**RHODOCHORTON VIOLACEUM (KÜTZ.) DREW, AND CHANTRANSIA BOWERI MURRAY AND BARTON.**—The species *Chantransia Boweri* was established by Murray and Barton,<sup>1</sup> who, while recognizing its close resemblance to *Rhodochorton violaceum* (Kütz.) Drew (then known as *Chantransia violacea* (Kütz.)), considered that the presence of hyaline hairs at the ends of the branches, the different proportions of the cells and the thinness of the cell-walls, were characters of sufficient importance to justify the separation of this alga from *R. violaceum*, as a distinct species. These investigators also described sexual reproduction for *C. Boweri*, and this constituted a more fundamental point of difference, since at that time only the monosporangia of *R. violaceum* were known. Recently the sexual reproductive organs of the latter species have been described<sup>2</sup> and, in addition, it has been found to possess an asexual tetrasporic generation. On account of considerable differences between the descriptions of the spermatangia branchlets and cystocarps of these two species, so alike in other respects, a re-examination of material of *C. Boweri* seemed desirable.

Thanks to the kindness of Sir Robert C. Mackenzie, the writer was able, in mid-August 1935, to visit the Cochno Burn, near Duntocher, Dumbartonshire, Scotland, the only locality where *C. Boweri* is known to occur. Information since received from Professor F. O. Bower, F.R.S., who was one of the collectors of the original material, leaves no doubt as to the exact identity of the locality visited with that of the earlier collection.

Many of the plants of the 1935 collection are reproducing sexually and a few by means of monospores only. Most of the cystocarps, which are numerous, are mature, and in some cases the carposporangia are already empty. It would appear that the collection was made too late in the season to obtain examples of the very first stages in the development of the cystocarp, as indeed would be expected from a study of *R. violaceum* in Derbyshire. Later stages have been seen, however, and the structure of these, as well as of that of the mature cystocarps, makes it clear that the cystocarps of the Scotch material develop in exactly the same way as those of *R. violaceum*. The only details which could be added to the account already given for *R. violaceum* are: (1) when the cystocarp is sessile, branches may develop from the cell bearing the cystocarp, no such branches having been seen in *R. violaceum*, and (2) cystocarps may terminate branchlets of more than three cells, i.e. the fertile branchlets may be longer than those recorded for *R. violaceum*.

Spermatangial branchlets are even more numerous than those bearing cystocarps. The cells of the main filaments of these plants do not decrease in size at

<sup>1</sup> Murray, G., and Barton, E. S.: On the structure and systematic position of *Chantransia* with a description of a new species. Jour. Linn. Soc. Bot., xxviii. 209-16, 1891.

<sup>2</sup> Drew, K. M.: The life-history of *R. violaceum* (Kütz.) comb. nov. (*C. violacea* Kütz.) Ann. Bot., xlix. 439-50, 1935.

[Annals of Botany, Vol. L. No. CXCVIII. April, 1936.]



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Many of the plants of the 1935 collection are reproducing sexually and a few by means of monospores only. Most of the cystocarps, which are numerous, are mature, and in some cases the carposporangia are already empty. It would appear that the collection was made too late in the season to obtain examples of the very first stages in the development of the cystocarp, as indeed would be expected from a study of *R. violaceum* in Derbyshire. Later stages have been seen, however, and the structure of these, as well as of that of the mature cystocarps, makes it clear that the cystocarps of the Scotch material develop in exactly the same way as those of *R. violaceum*. The only details which could be added to the account already given for *R. violaceum* are: (1) when the cystocarp is sessile, branches may develop from the cell bearing the cystocarp, no such branches having been seen in *R. violaceum*, and (2) cystocarps may terminate branchlets of more than three cells, i.e. the fertile branchlets may be longer than those recorded for *R. violaceum*.

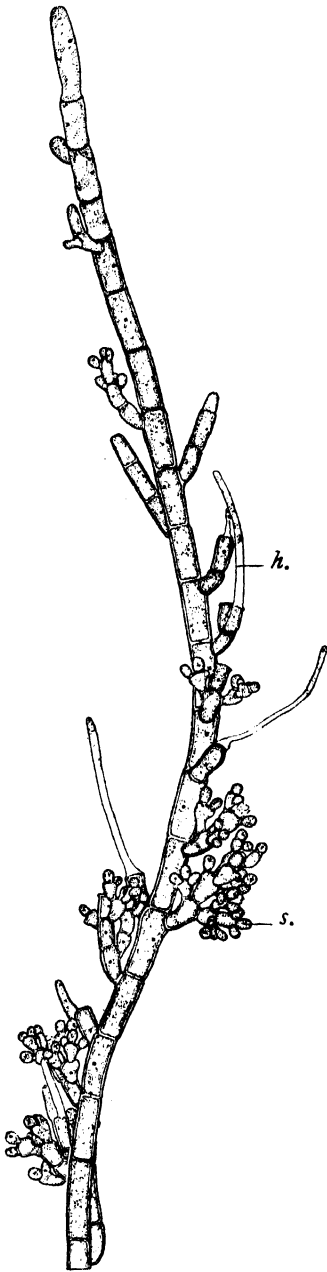
Spermatangial branchlets are even more numerous than those bearing cystocarps. The cells of the main filaments of these plants do not decrease in size at

<sup>1</sup> Murray, G., and Barton, E. S.: On the structure and systematic position of *Chantransia* with a description of a new species. Jour. Linn. Soc. Bot., xxviii. 209-16, 1891.

<sup>2</sup> Drew, K. M.: The life-history of *R. violaceum* (Kütz.) comb. nov. (*C. violacea* Kütz.) Ann. Bot., xlix. 439-50, 1935.

[Annals of Botany, Vol. L. No. CXCVIII. April, 1936.]

the apex, and the branchlets bearing the spermatangia are irregularly arranged.



Apical portion of male filament  
 s = spermatangium; h = hair.  $\times$   
 500.

Thus they differ from those described for *R. violaceum* from the River Goyt in Derbyshire, but are like the plant recorded from a tributary of the River Wharfe, Yorkshire l. c., p. 445). A re-examination of material from the River Goyt has however shown the occasional presence there also of male plants exactly like those from Scotland. It is conceivable that seasonal or environmental factors influence the type of male plant produced. The accompanying figure illustrates the type of male plant found in Scotland, although, for simplicity, a rather sparsely branched example has been chosen. Monosporangia occur occasionally on the spermatangial branchlets.

Just as there is no difference in the structure of the sexual reproductive organs of *C. Boweri* and *R. violaceum*, so there is no essential difference in size, the averages for the two plants being remarkably close.

As previously mentioned, Murray and Barton justified the separation of *C. Boweri* on points of difference of the monospore-bearing plants. Individuals bearing monosporangia are not common in the collection recently made and differ from those described by Murray and Barton, as the branchlets bearing the monosporangia are arranged irregularly and not in pairs or alternately. Moreover, the apical cells are not short, as they describe but, on the contrary, very long, as are those of *R. violaceum*. The difference in the branching may be a seasonal variation, as it has been found that the corresponding branchlets of *R. violaceum* sometimes develop in pairs in the spring.

The diameter of the cells of the asexual plants from Scotland is the same as of those of *R. violaceum*, but they are shorter, as Murray and Barton also found. A further investigation of the length of the cell of *R. violaceum* has shown that the cells of the spring and summer plants are both relatively and actually shorter than those of the winter plants. It is instructive to notice that the only measurements of *C. Boweri* are based on material collected in April and August, during which months the length of the cells of *R. violaceum* is similar to that of *C. Boweri*. Hence the

apparently shorter cells of *C. Boweri* would appear to be a seasonal and not a specific distinction. The other points of difference, accentuated by Murray and Barton, namely, the presence of hyaline hairs and the smaller diameter of the cell-wall, are very variable characters. In any case the material of *C. Boweri*, collected recently, is not marked by an abundance of hairs.

No tetrasporangia have been found on the Scotch plants, but since in the case of *R. violaceum* their appearance is confined mainly to the winter months, a collection made at the appropriate time of year might reveal their presence.

The method of attachment of the material from the Cochno Burn to the host (*Lemanea*) appears to be similar to that described for *R. violaceum*, and there is no evidence of non-septate rhizoids, as Murray and Barton describe.

This comparison between material of *C. Boweri* and *R. violaceum* would seem to show that there is every reason for uniting the two as one species. As *R. violaceum* is the older name, it is the one to be retained.

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November 1935.

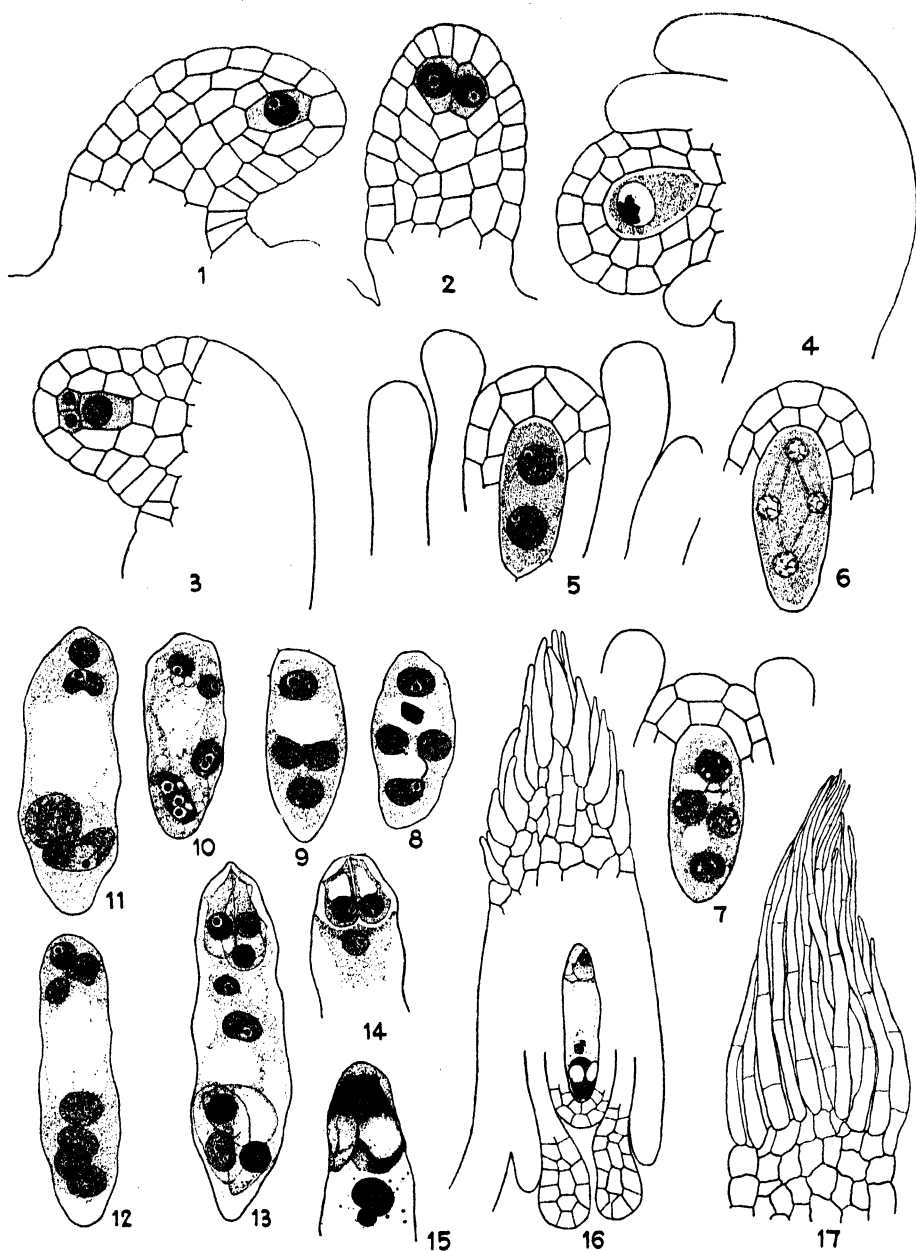
**A NOTE ON THE STRUCTURE AND DEVELOPMENT OF THE EMBRYO-SAC, OVULE, AND FRUIT OF *TAMARIX DIOICA* ROXB.**—In 1912 Frisendahl<sup>1</sup> described the development of the embryo-sac of *Myricaria germanica*, noting that it corresponds to the 'Lilium-type', but with many important modifications. For instance, he often observed both the chalazal nuclei in the 4-nucleate embryo-sac showing increase in size and number of chromosomes, mature embryo-sacs containing only one or two antipodals, and 4-nucleate embryo-sacs with one nucleus at the micropylar end separated by a large vacuole from three nuclei at the chalazal end. These observations have been recently re-interpreted by Schnarf<sup>2</sup> in the light of Bambicioni's<sup>3</sup> work on the embryology of *Fritillaria persica* as follows: 'The heterotypic division gives rise to two nuclei which occupy the whole cytoplasm of the embryo-sac (megaspore-mother-cell). Both the nuclei divide and give rise to the 4-nucleate embryo-sac. At this stage a large central vacuole develops and pushes three megaspore nuclei towards the chalazal end and one nucleus towards the micropylar end of the embryo-sac. During the next division, the three chalazal nuclei fuse while passing through the metaphase, anaphase, and telophase stages, and out of the original 4-nucleate embryo-sac again a 4-nucleate embryo-sac results which has two small haploid nuclei at the micropylar end and two large triploid nuclei at the chalazal end. Both the micropylar nuclei undergo one division and give rise to the egg-apparatus and the upper polar nucleus. Both the chalazal

<sup>1</sup> Frisendahl, A., Cytologische und entwicklungsgeschichtliche Studien an *M. germanica* Desv. Kungl. Svenska Vetensk. ak. Handl., xlviii, no. 7, 1912.

<sup>2</sup> Schnarf, K., Vergleichende Embryologie der Angiospermen., Berlin, 1931.

<sup>3</sup> Bambicioni, V., Ricerche sulla ecologia e sulla embriologia di *F. persica* L., Ann. di Bot., xviii. 7-37, 1928.





Figs. 1-17. *Tamarix dioica*. Various stages in the development of the ovule and the embryo-sac. Figs. 1-2. Young ovules showing primary archesporium, one-celled in Fig. 1, 2-celled in Fig. 2. Fig. 3. A young ovule showing a megaspore-mother-cell at an early stage of development and the parietal cell dividing anticleinally. Fig. 4. An ovule with megaspore-mother-cell in synizesis. Fig. 5. Micropylar portion of an ovule showing a 2-nucleate embryo-sac; the spindle-fibres of the previous division have not yet disappeared. Fig. 6. Micropylar portion of the nucellus of an ovule with a 4-nucleate embryo-sac just formed; the four nuclei are connected with one another by secondary spindle-fibres. Figs. 7-9. 4-nucleate embryo-sacs showing various stages of vacuolation

nuclei may not divide, or one or both may divide, so that a lower polar nucleus, and a variable number of antipodals, is formed.'

The present observations on the embryology of *Tamarix dioica* Roxb. have been made to determine whether other members of the family Tamaricaceae also show a similar peculiar development of the embryo-sac. For this purpose material was obtained from the local Ayurvedic garden, where some female plants of this species are cultivated.<sup>1</sup> It was studied according to the customary methods and the results agree with the above interpretation of Schnarf, as will be clear from the accompanying figures and the following description.

The sagittate staminodes in the female flowers of *T. dioica* never show any sporogenous tissue. The placentas inside the ovary are basal throughout their development. The ovules are anatropous, about  $225\ \mu$  long when mature, and possess two integuments, of which the inner projects beyond the outer and alone takes part in the formation of the micropyle (Fig. 16). The nucellus consists of two layers of cells (including the epidermis) around the embryo-sac. Both the integuments are two cells thick, except that the inner in its micropylar portion becomes three cells thick in its later stages. The primary archesporium appears before the initials of the integuments and consists generally of one (Fig. 1) but sometimes of two (Fig. 2) cells. A single archesporial cell develops further and it cuts off a parietal cell (Fig. 3), as Dahlgren<sup>2</sup> has observed in *T. tetrandra* and unlike what happens in *M. germanica*. The initials of the integuments appear about this time. The parietal cell soon after its formation divides in an anticlinal manner. The megaspore-mother-cell undergoes a fairly long period of rest, but ultimately undergoes the usual heterotypic division (Fig. 4), giving rise to two nuclei. No cell-plate appears at this stage, so that a 2-nucleate embryo-sac results (Fig. 5). The next division gives rise to four nuclei arranged in a bilateral fashion. These become connected together by secondary spindle-fibres forming the figure of a parallelogram (Fig. 6). As the spindle-fibres dissolve, vacuolation begins in the 4-nucleate embryo-sac. One or two (Fig. 7) vacuoles may begin to develop simultaneously. When there is only one, it separates three chalazal nuclei from one micropylar nucleus. When there are two vacuoles, one nucleus is separated at each end of the embryo-sac from two in the middle (Fig. 8). Later on the micropylar vacuole in such cases increases in size and the chalazal vacuole

<sup>1</sup> There are no male plants in the neighbourhood.

<sup>2</sup> Dahlgren, K. V. O., Die Morphologie des Nuzellus mit besonderer Berücksichtigung der deckzellosen Typen., Jahrb. f. Wiss. Bot., lxvii. 374-426, 1927.

and nuclei of equal size. Fig. 7 shows the beginning of vacuolation. Fig. 8 shows two vacuoles. Fig. 9 shows one large vacuole separating three nuclei at the chalazal end from one nucleus at the micropylar end of the embryo-sac. Figs. 10-11. 4-nucleate embryo-sacs with two small 1-nucleolate nuclei at the micropylar end and two large, mostly 3-nucleolate, nuclei at the chalazal end. Fig. 12. 8-nucleate embryo-sac, with four small nuclei at the micropylar end and four large nuclei at the chalazal end. Fig. 13. A mature embryo-sac, showing egg-apparatus, one small and one large polar nuclei and three large vacuolated antipodals. Fig. 14. Micropylar part of a mature embryo-sac, showing two egg-like synergids and the small upper polar nucleus. Fig. 15. Micropylar part of a degenerating embryo-sac, showing the disorganizing egg-apparatus, deposition of starch grains, and the two polar nuclei closely pressed against each other but still free. Fig. 16. Longitudinal section of an ovule containing a mature embryo-sac; the epidermal cells at the chalaza are beginning to grow. Fig. 17. Chalazal portion of a slightly older ovule showing a tuft of uni-seriate hairs. Figs. 1-15.  $\times 700$ . Figs. 16 and 17.  $\times 300$ .

is crushed, so that the ultimate result is the same, as in those embryo-sacs where there was only one vacuole from the beginning, and three nuclei are pushed towards the chalazal end and one towards the micropylar end of the embryo-sac (Fig. 9). Up to this stage all the four nuclei of the embryo-sac remain equal in size. During the next stage a 4-nucleate embryo-sac with two small nuclei at the micropylar end and two large nuclei at the chalazal end is formed (Figs. 10 and 11; Fig. 11 is a later stage and the nuclei are larger than in Fig. 10 and are preparing for the next division). The micropylar nuclei show mostly only one nucleolus, but the chalazal nuclei during this and the later stages contain mostly three nucleoli, showing that the large nuclei have resulted from the fusion of the three nuclei found at the chalazal end in the previous stage, just as Bambicioni has shown in *F. persica*. Next division takes place both in the micropylar and the chalazal nuclei, giving rise always to an 8-nucleate embryo-sac (Figs. 12 and 13). No variation has been observed in the division of the chalazal nuclei, as noticed by Frisendahl<sup>1</sup> in *M. germanica*.

The mature embryo-sac measures about 70  $\mu$  in length and shows the usual organization of an egg-apparatus, two polar nuclei, and three antipodals. The egg has the usual structure. The synergids develop small indentations in their upper part, but a 'filiform-apparatus' was not observed at their apices. They are peculiar in that they sometimes show egg-like vacuolation, i.e. their vacuole is situated towards the micropylar end and the nucleus with the cytoplasm is pressed against the chalazal end (Fig. 14). Such egg-like synergids have also been observed by Frisendahl in *M. germanica*, as may be seen from his Fig. 88 of Plate III, but on the whole they are very rare among flowering plants. Osterwalder<sup>2</sup> has noted such synergids in *Aconitum Napellus*, Persidsky<sup>3</sup> in *Delphinium elatum*, Puri<sup>4</sup> in *Moringa oleifera*, and Joshi and Rama Rao<sup>5</sup> in *Gisekia pharnaceoides*. These are all the examples that the writers have been able to collect. The antipodals are at first small, but ultimately they become larger than the cells of the egg-apparatus and develop large vacuoles. Their nuclei are larger than those of the egg-apparatus and the upper polar nucleus and commonly show three nucleoli. The lower polar nucleus has always been found to be larger than the upper and agrees in its size and structure with the nuclei of the antipodals. Both the polar nuclei gradually approach each other and come to lie below the egg-apparatus. In later stages they even come to closely press against each other, but they were not observed to fuse. Frisendahl has also noted this condition in *M. germanica* and says that the polar nuclei do not fuse till fertilization.

As there are no male plants of *T. dioica* in the neighbourhood of the female plants from which the material for the present study was collected, no pollination

<sup>1</sup> Frisendahl, A., Cytologische und entwicklungsgeschichtliche Studien an *M. germanica* Desv. Kungl. Svenska Vetensk. ak. Handl., lxxviii, no. 7, 1912.

<sup>2</sup> Osterwalder, A., Beiträge zur Embryologie von *A. Napellus* L., Flora, lxxxv. 254-92, 1898.

<sup>3</sup> Persidsky, D., Einige Fälle anomaler Bildung des Embryosackes bei *D. elatum*. Mem. Soc. Nat. Kiew, xxiii, 97-112, 1914.

<sup>4</sup> Puri, V., Note on the embryo-sac and embryo of *M. oleifera*. Proc. Ind. Acad. Sci., B, i. 279-82, 1934.

<sup>5</sup> Joshi, A. C., and V. Rama Rao, The embryology of *G. pharnaceoides* Linn., Proc. Ind. Acad. Sci., B, ii, 1936.

and consequently no fertilization takes place. The embryo-sacs degenerate after some time and no seeds with embryos are formed, but the ovules develop some characters of the seeds. The epidermal cells at the chalazal end of the ovule elongate (Fig. 16), divide, and develop into a tuft of long multicellular uniseriate hairs (Fig. 17) which are a characteristic feature of the seeds in the genus *Tamarix*. The ovary also begins to increase in size and develops into the fruit, which ultimately dehisces by three valves like the normal fruits, dispersing the plumed seeds, although these are without embryos.

Starch is deposited in the embryo-sac and cells of the nucellus, testa, and pericarp, as the embryo-sac degenerates (Fig. 15). This is probably correlated with the non-development of the embryo.

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# ***Aegilotriticum ovata-turgidum* a Fertile Species Hybrid.**

BY

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With Plate VI and twenty-three Figures in the Text.

EXPERIMENT has shown that all races of wheat (*Triticum*) cross more or less readily with almost all species of *Aegilops*, and in recent years numerous researches upon these hybrids have been carried out by many workers.

To such hybrids the convenient generic term *Aegilotriticum* has been applied by Dr. R. Wagner.

The majority of these hybrids are quite sterile, but in 1920-1 Tschermak (3) obtained a few grains in the ears of the hybrids *Aegilops ovata* × *Triticum dicoccoides* and *A. ovata* × a violet-grained wheat from Abyssinia.

The plants of the  $F_2$  generation raised from the hybrid grains, were found to be quite similar in morphological characters to those of the  $F_1$  generation, and successive fertile generations exhibiting the same constant characters, have been grown annually down to the present time.

In 1926 (2) I obtained a similar fertile cross between *A. ovata* ♀ and a variety of *Triticum turgidum* ♂. (*T. turgidum* var. *iodurum*. Poulard d'Australie).

Later, Kihara and Katayama (1) also described a fertile hybrid *T. dicoccoides* var. *Kotschyannum* ♀ × *Aegilops ovata* ♂.

The single plant of my hybrid was grown in the greenhouse and produced five or six tall culms with quite sterile ears, and later three shorter weak culms in the ears of which were four well-developed grains.

From the latter, two plants (the  $F_2$  generation) were raised in 1928, one of them producing typical hybrid ears like those of the  $F_1$  plant, but quite sterile. The other plant was attacked with wireworm (*Agriotes*) in the soil, and almost destroyed; it recovered, however, and although quite similar in appearance to the sterile ears of the sister plant, ripened several good grains.

A comparative description of the morphological characters of the parents and the hybrid are given below.

[*Annals of Botany*, Vol. L. No. CXCIX. July, 1936.]

	<i>A. ovata.</i>	Hybrid. (F <sub>1</sub> plant.)	<i>T. turgidum</i> , var. <i>iodurum</i> . (Poulard d'Australie.)
Young leaves.	Blade with scattered long hairs on the ridges; auricles and sheath fringed with long hairs.	Blade with long and short hairs; auricles and sheath with long hairs.	Blade with short velvety hairs; auricles with long hairs; sheath glabrous.
Culm.	35-40 cm. long; upper internode hollow, with thin walls.	60-5 cm. long; upper internode hollow, with thick walls.	130-5 cm. long, upper internode solid or hollow, with very thick walls.
Inflorescence.	3-4 cm. long; disarticulating at lowest node, leaving abortive spikelet attached to culm; rachis very tough, internodes 5-10 mm. long, scabrid; awns spreading.	6-9 cm. long, disarticulating as <i>A. ovata</i> ; rachis fragile, breaking easily at each node; internodes 7-8 mm. long, margins fringed with hairs; awns erect.	7-9 cm. long, rachis tough; internode 3 mm. long, smooth, fringed with long hairs; awns long, erect.
Spikelets.	3-5; each 15 mm. long, 1-2 lowest rudimentary, upper with 4 flowers, 2 fertile.	9-12; each 15 mm. long, lowest sometimes rudimentary, others with 6 flowers, some fertile.	23-4; each 9 mm. long, lowest 1-3 abortive, others with 3-4 flowers, 2-4 fertile.
Empty glume.	Pale straw colour; 10 mm. long, 7-8 mm. wide, thick, tough, without keel, but with 9-10 scabrid ribs; awns 4-5, scabrid, one short, the rest 2.5-4 cm. long in the upper spikelets.	Reddish-black, hairy, 9-10 mm. long, 5 mm. wide, thick, tough, with scabrid keel, ending in a scabrid awn 4-5 cm. long; lateral nerve ending in an awn 2-3 cm. long. A third median tooth is usually present.	Dark reddish-grey, very hairy, 7 mm. long, 4 mm. wide, thin, keel prominent, ending in tooth 1 mm. long, lateral nerve prominent; shoulder sloping.
Flowering glume.	10 mm. long, glabrous, with hairs at tip and margins, 5-6 nerves; awns 2 and one short tooth, one awn usually 2-3 cm. long, the other 1-2 cm. long.	9 mm. long, glabrous, with hairs at tip and margins, and a scabrid median awn 5-8 cm. long, with 2 lateral teeth.	8-9 mm. long, upper part and margins hairy, awn 8-10 cm. long, scabrid.
Palea.	Thin, awnless, bicarinate.	Thin, awnless, bicarinate.	Thin, awnless, bicarinate.
Grain.	7-8 mm. long, 3 mm. wide.	10-11 mm. long, 3.5-4 mm. wide.	6-7 mm. long, 3.5-4 mm. wide.

Comparison of the parents and the hybrid shows that the following characters belonging to *A. ovata* are dominant: hairiness of leaf-sheaf, and mode of disarticulation of the ear at the first node, resulting in the fall of the ear from the culm when ripe.

In the kind of hairs on the leaf-blade, height of the culms and thickness of their walls, number of spikelets, colour of the empty glumes, length of the chief awns of the flowering glumes, the hybrid is more or less an intermediate blend of the parents.

On the other hand, the grains and empty glumes of the hybrid are considerably longer than those of either parent, and a character possessed by neither parent is the fragile axis of the ear, which breaks readily at each node.

F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, and F<sub>6</sub> generations have been raised, the last in 1935.

In the F<sub>4</sub> generation two distinct types of plants were segregated, namely:—

(1) A lax-eared form (Pl. VI, Fig. 3, *a*, *b*) with dark brown or black glumes much darker than those of the F<sub>1</sub> plant. Some of these plants were quite sterile with very brittle axes breaking at the nodes, all the spikelets then separating from each other; others similar in appearance had fertile ears with tough axes, and well-developed grains in almost all the spikelets.

(2) A dense-eared type with pale straw-coloured or reddish glumes, sometimes with a tinge of green (Pl. VI, Fig. 4, *b*, *c*).

In the F<sub>5</sub> generation, plants were raised from the lax, black-eared, and the pale dense-eared types; the progeny of both exhibited the characters and fertility of their respective parents.

In the F<sub>6</sub> generation, plants from the lax, black-eared type again proved constant in morphological characters and high fertility, but among the progeny of the pale dense-eared fertile form F<sub>5</sub>, were many plants resembling the parent in appearance, most of them quite sterile, and plants with small, lax, sterile ears, some with pale reddish, others with dark brown glumes.

Another segregate of this generation was a dwarf plant about 30 cm. high, with narrow, dark green leaves having the velvet-like hairs of *T. turgidum*; the ears were of the ordinary hybrid type, small, dark-glumed and sterile.

Out of this cross *A. ovata* × *T. turgidum*, have arisen two well-defined segregates, namely:—

(1) Plants with lax, black-glumed ears, constant in morphological characters, usually quite fertile.

(2) A pale-glumed, dense-eared form also constant in its characters, but usually less fertile than (1).

The colour of the glumes of the first is derived from the wheat parent whose ears have traces of black pigment.

The glume colour of the second segregate is a pale straw colour like that of the *Aegilops* parent.

In most seasons individual plants of both forms are seen which are quite sterile, this being the only character in which there is still considerable variability; but for this feature these segregates might well be considered new species produced by hybridization.

In a previous communication (2) I gave an account of the results of an examination of meiosis in the pollen mother-cells of the F<sub>1</sub> hybrid plant,



which was found to be a diploid, having the normal twenty-eight univalents, namely, a set of fourteen from *A. ovata*, and fourteen from *T. turgidum*.

Investigation of the meiotic phase of the sterile and fertile  $F_2$  plants raised from the seeds of the  $F_1$  generation, was also made. It was discovered that both these plants were amphidiploid, possessing fifty-six chromosomes or twenty-eight bivalents in the pollen mother-cells. Doubling of both the *Aegilops* and the *Triticum* chromosomes had taken place, apparently during meiosis in the ears on the short, weak straws of the  $F_1$  plant sent up late; for an examination of the pollen mother-cells in the ears of the early, tall straws of the same plant, revealed only twenty-eight chromosomes present.

This year (1935) an examination was made of the chromosomes in the somatic cells (root tips), and in the pollen mother-cells of the parents of the cross, as well as in those of the two distinct types of segregated plants of the  $F_2$  generation.

The numbers in the somatic cells of the root tips were as expected, namely, fourteen in *A. ovata*, fourteen in *T. turgidum*, and twenty-eight in the amphidiploid hybrid. Text-figs. 2-4.

Polar views of the metaphase of the first division of the pollen mother-cells of the parents and hybrid, are illustrated in Text-figs. 5, 7, 9, and lateral views of the same in Text-figs. 6, 8, 10.

In both parents fourteen typical parasynthetic bivalents are present, while in the hybrid twenty-eight bivalents are seen, the majority acrosynthetic, the individual chromosomes of each pair being united end to end, with a small, variable number of the parasynthetic or ring type. Text-figs. 10, 11, 12, 14.

It is important to note that twenty-eight bivalents were found in the pollen mother-cells of sterile and fertile ears alike, of both types of segregates; the same number was found also in mother-cells of the sterile dwarf plant of this generation.

In one or two instances, pollen mother-cells with only fourteen bivalents were observed among the cells of pollen sacs in which twenty-eight bivalents was the prevailing number. Text-fig. 13.

In the anaphase of the first division, the univalents divide longitudinally, as in all hybrids of *Aegilops*  $\times$  Wheat hybrids, and although irregularities are sometimes observed, the number of the resulting monads reaching the poles are usually equal, few 'lagers' being seen.

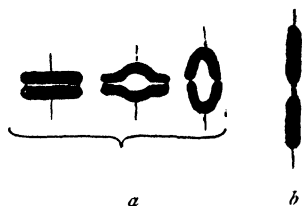
The homotypic, or second division of the pollen mother-cells was found to follow the normal course, the individual cells of the tetrad containing twenty-eight chromosomes.

Meiosis in this hybrid, is in close agreement with the meiotic phases in the fertile *A. ovata*  $\times$  Wheat hybrids obtained by Tschermak and Kihara and Katayama.

While successive generations of these hybrids exhibit constant morphological characters, considerable variation in respect of fertility of their ears and individual plants is met with in all of them.

I have grown eight generations of Tschermak's two hybrids and find that these show irregularities in their fertility similar to those observed in my hybrid segregates; Tschermak's cross *A. ovata* × wild *T. dicoccoides*, varies much more in fertility than his hybrid *A. ovata* × violet-grained Abyssinian cultivated wheat.

In regard to the pairing of univalents at metaphase of the heterotype division of pollen mother-cells, two distinct types of union are found, namely, (1) parasyndetic, or side by side pairing, the two conjugating chromosomes being closely associated along their whole length, or united at both ends to form a link or ring, which is arranged at right angles to the spindle fibres; and (2) acrosyndetic, or end to end pairing, in which the two univalents are attached to each other at one end only, the bivalents thus formed being arranged along the spindle fibres. Fig. 1.



TEXT-FIG. 1. Types of bivalents as seen at metaphase of heterotype division. *a.* parasyndetic bivalents; *b.* acrosyndetic bivalent characteristic of species hybrids.

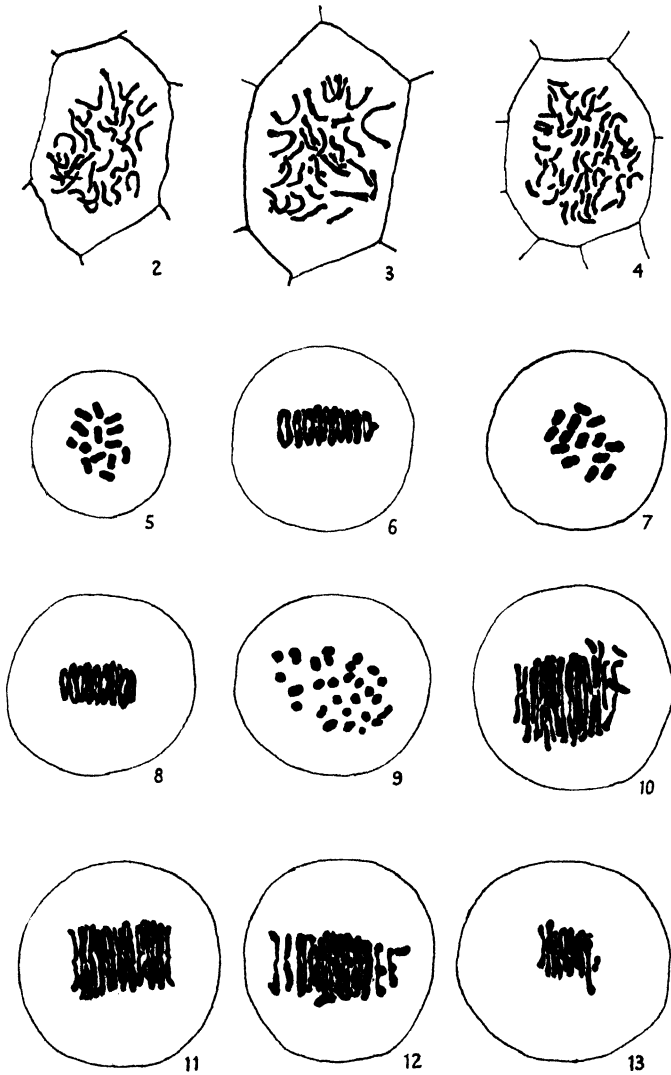
Where univalent chromosomes are longer than broad, metaphases with acrosyndetic or parasyndetic bivalents can be recognized with certainty at a glance (cf. Text-figs. 6 and 10); of course, where the univalents are spherical the type of union cannot be determined.

Metaphases in which all the bivalents are of the parasyndetic type, as in Text-figs. 6, 7, are characteristic of all self- or cross-fertilized plants of the same species, while metaphases with the acrosyndetic type of bivalent are only found in plants which are hybrids.

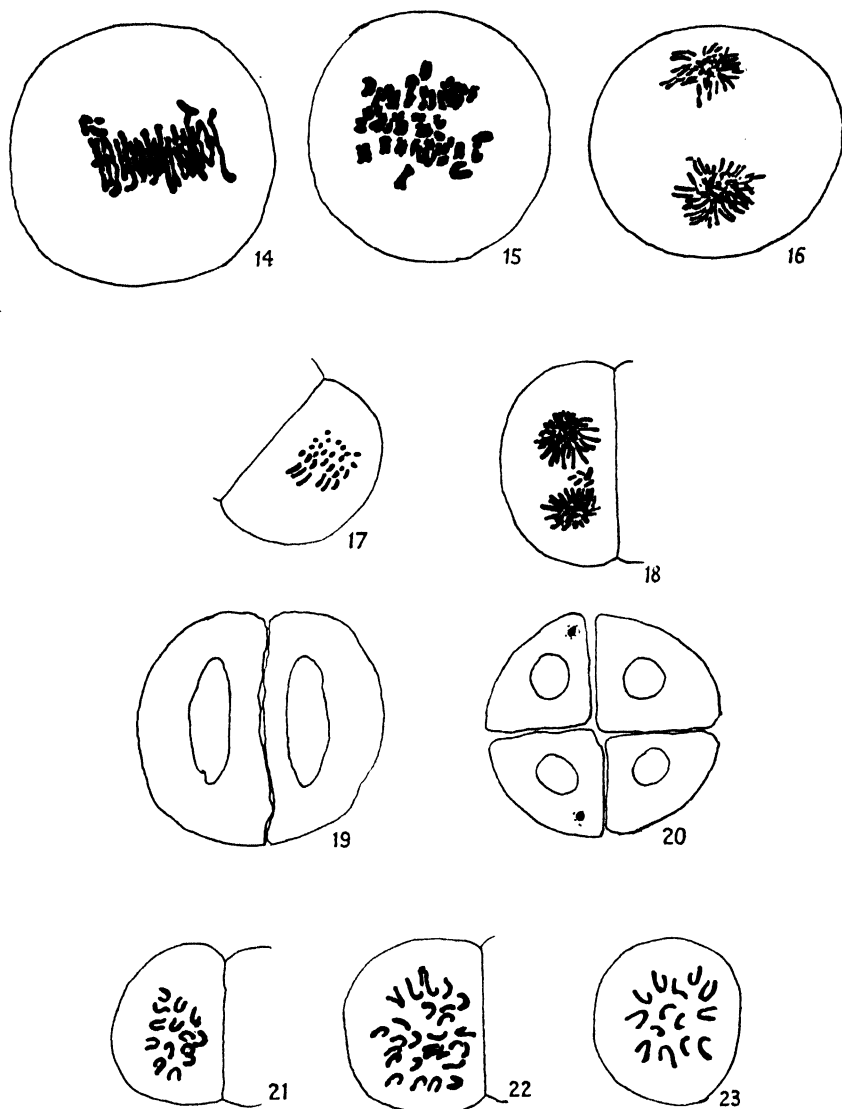
In a previous communication (2) I expressed the view that parasyndetic bivalents as seen in metaphases of the heterotype division are only found when the uniting univalents are exactly homologous, and that end to end pairing is evidence of more remote relationship between the conjugating chromosomes, such as that between less closely related species or subspecies. In hybrids in which there is no pairing, the univalents belong to different species.

Thus the absence of pairing, or the type of union among univalents at the metaphase of the heterotype division may be used as a measure of homology of the conjugating chromosomes. Examination of published figures of heterotype metaphases of pure species and of hybrids, show that these conclusions are of very wide application.

That amphidiploid hybrids with double sets of parental chromosomes are fertile and breed true, is generally attributed to regular pairing between homologous chromosomes from the similar parental sets, and the formation



TEXT-FIGS. 2-13. Fig. 2. Metaphase plate of somatic chromosomes (root-tip cell) of *A. ovata*. Fig. 3. do. of *T. turgidum* var. *iodurum*. Fig. 4. do. of hybrid *A. ovata* × *T. turgidum* var. *iodurum*. Figs. 5 and 6. Heterotype metaphase of *A. ovata*, polar and profile views respectively. Figs. 7 and 8. do. of *T. turgidum* var. *iodurum*. Figs. 9 and 10. do. of hybrid. Figs. 11 and 12. Profiles of heterotype metaphases of the hybrid. Fig. 13. Profile of heterotype metaphase of a pollen mother-cell with 14 (?) bivalents instead of 28; from the same pollen sac of the hybrid as Figs. 10 and 11.



TEXT-FIGS. 14-23. Fig. 14. Profile of heterotype metaphase of hybrid. Fig. 15. Metaphase of hybrid showing early division of the univalents. Fig. 16. Late heterotype anaphase of hybrid. Fig. 17. Polar view (slightly oblique) of hybrid homotype anaphase group, showing 38 chromosomes. Fig. 18. Profile of late anaphase of homotype division in hybrid. Fig. 19. Dyad of hybrid. Fig. 20. Tetrad of hybrid. Figs. 21 and 23. Metaphase plate of homotype division of *A. ovata* and *T. turgidum* respectively, showing 14 chromosomes. Fig. 22. do. of the hybrid showing 28 chromosomes.

of gametes to which an equal number of chromosomes have been distributed.

Since the microspores of both fertile and sterile plants of these amphidiploid *Aegilops* × *Wheat* hybrids possess the same number of chromosomes, namely, 28, it seems clear that it is not the number of chromosomes in the gametes which controls fertility; an explanation of fertility and sterility is rather to be sought in the kind of chromosomes in the gametes, and this is dependent upon the nature of the univalents which conjugate to form the bivalents seen at the metaphases of the heterotype division.

It is concluded from an examination of the cytology of the reproductive cells of pure species, that the bivalents of the heterotype metaphases are formed by the conjugation of two exactly homologous univalents.

While the presence in these amphidiploid hybrids of two complete sets of the chromosomes of each parent, makes it possible for the formation of homologous bivalents during meiosis, nevertheless, metaphases showing only parasyndetic association, such as is always observed in the heterotype division of pure species, are never found.

A variable number of bivalents of the parasyndetic type are seen, but the majority are of the acrosyndetic, or end to end type, which according to the view expressed above, are made up of a single univalent from each parent.

Such acrosyndetic bivalents formed by amphidiploid plants, may in some instances give rise to gametes approximating very closely to those which would be produced if all the bivalents were of the parasyndetic type: for random orientation of the end to end bivalents at metaphase of the heterotype division, would be likely to result in an equal or almost equal number of the *Aegilops* and *Triticum* halves of the bivalents facing towards the same pole of the mother-cell.

Where orientation was such as to lead to the distribution of very unequal numbers of *Aegilops* and *Triticum* chromosomes towards the same pole, complete sterility might be expected.

Possibly the segregation into two types—namely, the two more or less fertile types, one with glumes of the pale straw colour like those of the *Aegilops* parent, the other with darker glumes associated with the *Triticum* parent—may be due to a slight excess of *Aegilops* or *Triticum* chromosomes in the gametes, the differences being not sufficient to induce complete sterility.

#### SUMMARY.

1. A description is given of the morphological characters of a fertile amphidiploid hybrid *Aegilops ovata* × *Triticum turgidum* var. *iodurum*. Some of the *Aegilops* characters were dominant in the  $F_1$  plant, while in other characters the plant was an intermediate blend of the two parents.

2. The hybrid has been grown for six generations, during which period there have appeared two distinct types of plants, one of them with glumes of a pale straw colour like those of the *Aegilops* parent, the other with black glumes, their pigment derived from the *Triticum* parent.

While both types are usually fertile, individual plants are found in each generation which are sterile; fertility is a variable character.

3. In two characters, namely, length of empty glume and size of grain, the hybrids exceed those of either parent.

4. Most of the ears of the  $F_1$  plant were sterile, and the pollen mother-cells diploid, having twenty-eight chromosomes, fourteen from the *Aegilops*, and fourteen from the *Triticum* parent. Four grains, however, were ripened in three ears on very short straws of the plant, and the  $F_2$  descendants raised from these, some sterile others fertile, all contained in their pollen mother-cells, fifty-six chromosomes, doubling of both parental sets having taken place apparently in the reproductive cells of some of the fertile ears of the  $F_1$  plant.

5. An examination was made of the number of chromosomes in the somatic cells of the root-tips and in the pollen mother-cells, both of the parents and the amphidiploid plants of the sixth generation.

6. The bivalents at the metaphase of the heterotype division of the parents were of the characteristic parasynthetic type like those of all pure species whose chromosomes are longer than broad; those of the hybrids were chiefly of the acrosynthetic, or end to end type, usual in *Aegilops* × *Triticum* hybrids.

7. In the anaphase of the first division of the pollen mother-cells of the hybrid longitudinal splitting of the univalents occurs, the second division following the normal course, each cell of the tetrads receiving twenty-eight chromosomes.

8. Reference is made to the problem of fertility in these amphidiploid hybrids.

I take this opportunity of thanking Professor Brierley, for the provision of facilities for carrying out this work in the Department of Agricultural Botany, The University, Reading.

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## EXPLANATION OF PLATE VI

Illustrating Professor Percival's paper on '*Aegilotriticum ovata-turgidum*: a Fertile Species Hybrid'.

Figs. 2, 3, and 4 were drawn with the aid of Abbe's camera lucida from permanent preparations (Zeiss apochromat 2 mm. objective and No. 12 ocular; the rest from fresh preparations stained with aceto-carmin (Zeiss apochromat 2 mm. objective and No. 8 ocular).

FIG. 1. *a*. Ear of *Aegilops ovata*; *b*. Ear of *Triticum turgidum* var. *iodurum*; *c*. Ear of hybrid *A. ovata* × *T. turgidum* var. *iodurum*.

FIG. 2. *a*. Empty glume, flowering glume and grains of *A. ovata*; *b*. do. of *T. turgidum*; *c*. do. of the hybrid.

FIG. 3. *a*. Ear of lax-eared, black-glumed fertile hybrid, with glumes and grains; *b*. ear of lax-eared, black-glumed sterile hybrid segregate.

FIG. 4. *a*. Pale-glumed fertile hybrid segregate; *b*, *c*. Dense-eared, pale-glumed fertile hybrid segregates.







# The Concentration of Solutes in Sap and Tissue, and the Estimation of Bound Water.<sup>1</sup>

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## I. INTRODUCTION.

OBSERVATIONS (9) made in this laboratory on the relation between the sugar gradients on the one hand, and the rate and direction of carbohydrate transport on the other, have suggested that the mechanism responsible for longitudinal movement through the sieve-tube system resembles the process of physical diffusion in that rate and direction of transport are determined by the sugar gradients, but that the rate is greatly

<sup>1</sup> Paper No. 14 from the Physiological Department of the Cotton Research Station, Trinidad.  
[*Annals of Botany*, Vol. L. No. CXCI. July, 1936.]

accelerated by some unknown agency. Transport appears in short to proceed by a process of *activated diffusion*, which may be fundamentally akin to secretion (11). Further work on the gradients of nitrogenous substances and on movement has on the whole tended to re-enforce this suggestion. The work has been extended to include the mineral elements, but as yet investigation (7, 10) has been limited to ascertaining the elements that are mobile in the phloem and to elucidating the gross phenomena of movement.

In order to carry the analysis a stage further, it was decided to examine the concentrations of the sap-soluble fractions of the various elements. Following our usual procedure, the sap concentrations were determined on sap expressed from previously frozen tissues. From these concentrations, assuming that the water remaining in the tissue contains the same concentration of solutes as the expressed sap, and a knowledge of the total water content of the sample, it is possible to calculate the weight of any element in solution. For an element completely sap-soluble the weight so determined should equal that obtained by direct estimation on fresh material (or, if more convenient, on dried material), while for elements not entirely soluble the direct estimate should exceed the sap estimate.

In the course of preliminary work it was found that the sap estimate for chlorine exceeded the direct estimate by about 10 per cent. Re-examination of the estimates for sugar furnished by the two methods, which we have recently published (15), has shown them to exhibit the same tendency. The obvious inference from these observations is that the water remaining in the tissue after pressing could not have had the same concentration of solutes as the expressed sap. Either the method used for the expression of sap was faulty or a part of the unexpressed water was not functioning as a solvent. The work reported in the present paper was carried out in order to ascertain the cause of the difference in the two estimates for chlorine and for sugar, and has been extended to include calcium, magnesium, and potassium.

## II. SUGAR AND CHLORINE CONCENTRATIONS IN SAP AND TISSUE.

### (A) *Methods.*

The bulk of the work reported in this paper was carried out on the leaf of the cotton plant (*G. barbadense* L.), but some observations were also made on the leaves of other plants.

#### (1) *Analytical.*

The materials estimated include sugars, calcium, magnesium, potassium, and chlorine. The methods have been described in previous papers, but are summarized here for convenience. *Sugars* (15): the reducing power

of all sugars was determined by the Shaffer-Hartman method. Sucrose was determined by the increase in reducing power after inversion by invertase. Total sugars represent the total sugars found after inversion. *Calcium* (10) was estimated as the oxalate and *Magnesium* (7) as magnesium ammonium phosphate after removal of calcium. For *Potassium* (5, 10) the cobaltinitrite method, and for *Chlorine* (7) Volhard's method, were employed.

(2) *Clearing.*

*Procedure.* Sap was expressed from previously frozen tissue and allowed to sediment. The procedure followed for expression of sap and the method of clearing the sap for the estimation of sugar have already been described (15). For the mineral elements, which may exist in the sap partly as adsorption compounds with the sap colloids, and may be partly in true solution either as organic and/or as inorganic compounds, it is necessary to resort to other methods of clearing. As we require only substances in true solution, and as we ought to know the extent to which they are present in an ionizable form, the following procedure was adopted:

The estimation of elements present in the ionizable state necessitated analysis on *unashed* sap. For ease of working, it was found desirable to remove colouring matter. For this purpose we used charcoal in acid solution. Emmert (3), who employed this method for the estimation of phosphate, used 5 gm. of charcoal for each gramme of tissue, and after thorough grinding together added 50 ml. of 1 per cent. sulphuric acid and filtered after five minutes.

We have used a commercial decolorizing charcoal known as Suchar, and have found that with this charcoal the proportion used by Emmert was higher than necessary. One gramme of this carbon completely decolorized 10 ml. of leaf sap almost instantaneously, while half a gramme acting for half an hour left some colour. Acid was used at the rate of 250 ml. of 1 per cent. sulphuric acid per 100 ml. of leaf sap. It was found that sulphuric acid of this concentration precipitated most of the calcium in the sap and so, for the clearing of sap for the determination of ionizable calcium, hydrochloric acid of equivalent strength was used.

The possibility of adsorption has been tested by using different proportions of charcoal and comparing the amounts of the various elements after *clearing and ashing* with those present in the *uncleared ashed sap*. Duplicate samples of 10 ml. portions of leaf sap were cleared with 1, 2, and 5 gm. of carbon in the presence of 25 ml. of acid and the whole filtered as rapidly as possible after thorough mixing. The filtrates were quite colourless in all cases. The charcoal was washed, the combined filtrates and washings made to standard volume, and aliquots taken for the determination of ionizable (unashed) and of total (after ashing) mineral elements.

*Results.* The results are shown in Table I. It will be seen that none of the elements examined appears to be adsorbed with 1 gm. of carbon, for the differences between the 'ashed' totals on the cleared (1 gm. carbon) and on the uncleared saps are in no case significant. For calcium, the mean *ionized* value exceeds the mean *total* value, but the difference is small and not significant. For magnesium and potassium, there is close agreement between the mean *ionized* and the mean *total* values for the cleared saps. A non-ionizable fraction for these elements is not therefore indicated.

TABLE I.

*Showing the Effect of Varying Amounts of Charcoal on the Concentration of Ionizable and of Total Elements in a Leaf Sap. (Concentrations in mg. per 100 ml. Sap. November 17, 1932.)*

Gm. charcoal per 10 ml. sap.	Calcium.		Magnesium.		Potassium.		Chlorine.	
	Ionized.	Total (ashed).	Ionized.	Total (ashed).	Ionized.	Total (ashed).	Ionized.	Total (ashed).
1	590	592	117	121	544	550	417	432
2	592	581	116	118	542	539	412	419
5	564	555	102	100	543	540	390	406
Mean	582	576	112	113	543	543	406	419
Uncleared	—	586	—	121	—	541	—	436
Significant differences {		$P = 0.05$	25.5	9.4	28.9	8.9		
		$P = 0.10$	20.0	7.4	22.9	7.2		

The estimation of chloride has usually given values lower than those for total chlorine. The mean chloride concentration in Table I was 406 mg., while the mean total chlorine concentration was 419 mg. As this difference is significant, it suggests that chlorine may be present in a non-ionizable form, but repeated attempts to detect it have proved abortive. The method employed consisted in the removal of all chloride from the sap with a small excess of silver nitrate and the removal of the excess silver nitrate with thiocyanate. The filtrate was then made alkaline with sodium carbonate and evaporated to dryness. After ashing, tests for chlorine have always yielded negative results. It seems probable that the differences between chloride and total chlorine are due to interference by colloids not removed by carbon in the precipitation of the silver chloride (cf. Warburg (19)). There would appear, therefore, to be no evidence for the presence of chlorine in the sap in any form other than chloride.

While the use of charcoal decolorizes the sap, it does not remove all colloids, some of which may carry adsorption compounds. Some of these compounds may be released in clearing and some may be released by the reagents used for the analysis. Thus the possibility exists that part of the potassium, for instance, may not be in true solution. There would appear to be no means of guarding against this contingency.

(B) *Concentrations.*

Column 1 of Table II contains the concentrations of chlorine and of sugars in the sap expressed from four collections of leaves. A collection consisted usually of two samples, but in Experiment 4 there were four samples. These concentrations depend only on the weight of material and weight of water found in a given volume of sap. The weight of water was determined by drying at 100° C. Complete results for the sugars are available only for Experiments 2 and 4.

TABLE II.

*Concentrations of Chlorine and Sugars (gm. per 100 gm. water) in the Expressed Sap, Whole Tissue and Pressed Residue.*

Experiment.	Date.	Material.	Sap.	Concentrations in	
				Whole tissue.	Residue.
			1.	2.	3.
1	Oct. 2, 1933	Chlorine	0.576	0.519	0.315
		Chlorine	0.164	0.140	0.123
2	Oct. 12, 1933	Total sugars	1.34	1.08	—
		Sucrose	0.51	0.41	—
3	Nov. 24, 1933	Chlorine	0.323	0.293	0.284
		Total sugars	1.12	0.96	—
		Chlorine	0.236	0.208	0.173
4	Mar. 5, 1934	Total sugars	1.82	1.57	—
		Sucrose	0.68	0.61	—

In column 2 of the table will be found the concentrations in the whole tissue. The chlorine concentrations depend on the weight of chlorine in, and the weight of water calculated from, material dried at 100° C. The weight of sugar was determined by extraction of fresh tissue with boiling alcohol. It will be seen that in every case the *concentration in the sap exceeds that in the whole tissue.*

In column 3 are shown the concentrations of chlorine found in the residue left after the expression of sap. They are obtained from the weights of chlorine in and the weights of water found on drying the residues to constant weight at 100° C. It will be seen that the *concentrations in the residue are much less than in the sap.* Sugar concentrations in the residue, using alcohol for sugar extraction, were also determined, but are not presented, as some hydrolysis of sucrose and of polysaccharides apparently took place (see Table III).

III. POSSIBLE CAUSES OF DIFFERENCES IN CONCENTRATION ESTIMATES.

(A) *Analytical Errors.*

Table III shows the balance sheet for Experiment 4 and from this data the concentrations given in Table II were derived. There is a difference

between the water per 100 gm. fresh weight as determined by drying at 100° C. and by alcohol extraction, followed by drying of the extract and residue at 100° C. In calculating the whole tissue concentration, the water value obtained by drying at 100° C. was used. If the alcohol value had been used instead, the concentrations of total sugars and of sucrose would be increased from 1.57 gm. and 0.61 (0.606) gm. to 1.59 gm. and 0.61 (0.612) gm. per 100 gm. water respectively.

TABLE III.

*Gm. Dry Weight, Water, Sugars, and Certain Mineral Elements in Expressed Sap and Pressed Residue, and Weights in the Whole Tissue found by Alcoholic Extraction and after Drying at 100° C. Results Expressed per 100 gm. Original Fresh Weight.*

*Experiment 4, March 5, 1934.*

	Dry weight.	Water.	Total sugars.	Sucrose.	Calcium.	Magnesium.	Potassium.	Chlorine.
Sap . . . . .	5.87	51.64	0.94	0.35	0.423	0.035	0.466	0.122
Residue . . . . .	21.44	19.67	0.37	0.00	0.443	0.030	0.268	0.034
Sap + Residue . . . . .	27.31	71.31	1.31	0.35	0.866	0.065	0.734	0.156
Whole tissue { Dried at 100° C. . . . .	27.34	72.66	—	—	0.852	0.064	0.751	0.151
{ Alcohol extract + residue . . . . .	28.15	71.85	1.14	0.44	—	—	—	0.159
Significant differences { $P = 0.05$ . . . . .	0.542	0.995	0.076	—	0.032	0.008	0.021	0.011

The water content of the sap + residue is significantly smaller than that of the whole tissue obtained by drying at 100° C., while the dry weights scarcely differ. This loss of water may have taken place, partly or even wholly, from the sap during its expression, in which case the sap concentration would be too high. Correction for this water loss reduces the sap concentration of total sugar and sucrose from 1.82 gm. and 0.68 gm. to 1.77 gm. and 0.66 gm. per 100 gm. water respectively. These corrected sap concentrations are higher than the corrected (alcohol) whole tissue concentrations by 11.3 per cent. for total sugars and by 7.8 per cent. for sucrose.

These water corrections also apply to the chlorine concentrations, but in addition there is a third correction to be considered, due to the variation in the three estimates of chlorine per 100 gm. fresh weight. The concentration in the whole tissue given in Table II was obtained, using the value 0.151 gm. per 100 gm. fresh weight as found by estimation on material dried at 100° C. Judging from the values found by alcoholic extraction and from the sap + residue, the value is low, although the differences are not significant.

If 0.156 gm. is taken as the correct value, and applying the water corrections as above, the concentrations of chlorine in the sap, whole tissue, and residue become 0.230, 0.215, and 0.173 gm. per 100 gm. water respectively. The differences in concentrations between the whole tissue and its constituent parts cannot, therefore, be explained on the grounds of analytical errors.

(B) *Sap Expression.*

When the sap is expressed from untreated leaf tissue, the first fraction of sap is usually more dilute than the last fraction. This is due to a difference in the permeability of protoplasm to water and solutes (2). If the reverse were to occur, as a result, for instance, of more ready sap expression from the veins than the mesophyll,<sup>1</sup> when sap is expressed from frozen material, it might explain the difference in chlorine concentration between the expressed sap and the pressed residue. It would appear, however, that all fractions of sap expressed from frozen material have the same concentration of solutes. Thus, Dixon (2) found that successive fractions had the same freezing-point depression and we (15) have shown that for cotton leaves the sugar concentrations remain unaltered. The sugar and chlorine concentrations in sap expressed from frozen leaves and the concentration in the residue after pressing are shown in Table IV. It will be seen that while the concentrations in successive fractions of the expressed sap show no significant change, there is a sudden break between the last fraction and the residue.

TABLE IV.

*Sap and Residue Concentrations Expressed in gm. per 100 gm. Water.*

	Chlorine.	Total sugars.
Sap { 1st fraction	0.324	1.17
2nd    ,,	0.327	1.17
3rd    ,,	0.331	1.18
Significant { $P = 0.05$	0.019	0.10
differences { $P = 0.10$	0.016	0.08
Residue	0.299	—

(C) *Changes in Water and Solutes Brought About by Experimental Procedure.*

(1) *Effect of drying on the estimation of water.*

The estimation of water in the whole tissue and in the pressed residue involves heating a mass of wet tissue to 100° C. It is possible that changes may take place under these conditions leading to loss of dry matter and consequent over-estimation of the water present. Such over-estimation would cause a reduction in the observed concentrations in the tissues. The

<sup>1</sup> The concentration of chlorine in the cotton leaf has been found to be much greater in the vein than in the mesophyll.



determination of water in the expressed sap would scarcely be affected by such considerations owing to the much greater proportion of water to organic matter. That there is little or no destruction of dry matter during the drying process is shown by the very small  $\text{CO}_2$  output, which is of the order of 0.1 per cent. of the fresh weight, and which is given off almost entirely during the first few minutes. Furthermore, drying at temperatures below  $100^\circ\text{C}$ . leads to water estimates only slightly below those obtained at  $100^\circ\text{C}$ . Thus, in Experiment 4, whole tissue dried to constant weight at  $50^\circ\text{C}$ . indicated a moisture content of 71.75 gm. water per 100 gm. fresh weight, a value only 0.91 gm. or 1.25 per cent. lower than that obtained at  $100^\circ\text{C}$ . Sayre and Morris (17), comparing methods of drying corn tissue, found that air drying at  $50^\circ\text{C}$ . indicated a value of 78.48 gm. water per 100 gm. fresh weight, whilst three other methods involving drying at temperatures close to  $100^\circ\text{C}$ . gave closely agreeing estimates with a mean of 79.07 gm. per 100 gm. fresh weight. It will be evident that drying at  $100^\circ\text{C}$ ., although an arbitrary procedure, is unlikely to lead to any serious over-estimation of the water content. On the other hand, there is some evidence that such drying may lead to an under-estimate. Thus, Nelson and Hulett (12) found that edestin dried at  $100^\circ\text{C}$ . *in vacuo* still retained at least 1.9 per cent. of water, and concluded that no biological material could be completely dried without destroying the organic structure.

(2) *Effect of freezing on the concentration of solutes.*

It is sometimes assumed that sap expressed from frozen tissue has the same solute composition as the sap in the intact untreated tissue. The possibilities that changes occur in concentration of individual solutes as a result of freezing does not appear to have been adequately investigated. It will be evident, that if the sugar concentration was increased as a result of freezing, the concentration in the expressed sap would exceed the tissue concentration based on alcohol extraction. On the other hand, the chlorine concentration should not be altered by freezing. There would appear to be three methods of determining the effect of freezing the tissue on the solute concentration of the expressed sap.

Firstly, there is the comparison of the osmotic pressure of the tissue by the plasmolytic and cryoscopic methods. This comparison does not appear to have been done with adequate precaution, but Oppenheimer (14) has compared the osmotic pressure of twelve species of plants by the plasmolytic and cryoscopic methods, using sap obtained from tissues heated to  $100^\circ\text{C}$ . He corrected for temperature and the diminution in volume from turgidity to flaccidity. Though he found good agreement, it must be emphasized that transformation, especially of carbohydrate, is liable to occur with heat.

The second method depends on the comparison between the solutes obtained in sap expressed from previously frozen tissue and from tissues extracted with some solvent (e.g. alcohol for sugars and chlorine). Sayre and Morris (18) have found that the weight of sugar in corn (*Zea Mays* L.) leaf tissue could be determined either by the sap or the alcohol method. Their results, therefore, differ from ours with cotton, for we have found that the sap method yields higher results. If we accept their results, it should follow that the concentration of sugar in the sap expressed from corn leaves is the same as that in the sap of the intact plant. They also expressed sap from ground tissue, and claim that the sugar concentration is the same as in sap expressed from frozen tissue.

Thirdly, the composition of sap expressed from frozen tissue may be compared with the sap obtained from tissues rendered permeable by some other means. Thus, if it could be demonstrated that the solute composition of the sap was independent of the method of destroying permeability, there would be grounds for the belief that the expressed sap was a representative sample of the sap in the tissue. We have, therefore, compared the composition of the sap expressed from frozen tissue and from tissue treated with toluene. Toluene appeared to us preferable to other cytolysing agents owing to its low solubility in water. It would appear that hitherto only the freezing-point depression of frozen and toluene-treated tissues has been compared (8). Sap expressed from leaves treated with toluene has been found to have a lower osmotic pressure than sap expressed from frozen leaves. It appeared to us that this might well be due to loss of sugar as a result of the stimulation of respiration by the toluene, and that the concentration of some or all of the other solutes might be unaffected. We have, therefore, compared the concentrations of certain of the mineral elements and total sugar in sap obtained by the two methods.

The results of an experiment designed to test this point are shown in Table V. The various treatments used are described in the table. Duplicate samples were employed throughout. For the toluene treatment two weighed samples were each placed in a vacuum desiccator, which was exhausted at intervals of half an hour, and refilled with air saturated with toluene vapour. After four hours the leaves were removed, weighed, and the sample subdivided for moisture determination and sap expression. The loss in dry weight amounted to 4.96 per cent. and considerable heating occurred. The sap concentrations recorded in the table are corrected for moisture changes during the toluene treatment; the correction amounted to 3.52 per cent.<sup>1</sup>

It will be seen that the mean values for  $-15^{\circ}\text{C.}$  are consistently

<sup>1</sup> The correction should more strictly be applied to the free water content of the tissue (cf. IV), and would then amount to 4.29 per cent. For the present purpose it is immaterial which correction is used.

greater than those for  $-5^{\circ}\text{C}$ . The latter temperature was, therefore, insufficient to render these leaves completely permeable. The differences between the four and the twenty-four hour periods at  $-15^{\circ}\text{C}$ . are not consistent, and except for calcium are not significant. The difference for calcium just attains the level of partial significance. Comparing the values for toluene and the mean values for  $-15^{\circ}\text{C}$ ., it will be noticed that the differences for magnesium, potassium, and chlorine are small and not significant, but that total solids, calcium, and sugars are significantly less for the toluene treatment. Less sap (water) was obtained from the tolued leaves.

Another experiment was carried out along the same lines, but corollas were used in place of leaves and samples were frozen with carbon dioxide as well as at  $-15^{\circ}\text{C}$ . The dry weight loss in this case was only 0.93 per cent, and the moisture correction was 1.32 per cent. The results, which are shown in Table VI, confirm those of Table V. No differences are shown between the samples frozen at  $-15^{\circ}\text{C}$ . and with carbon dioxide. The differences between the frozen and the toluene-treated corollas do not attain the level of partial significance for magnesium, potassium, and chlorine, but are fully significant for calcium and sugars. The difference for total solids, though in the same direction as the last experiment, is not, however, significant. Once more, the volume of sap from toluene-treated material was much smaller than from frozen material.

The reason for the difference in the amount of sap (water) obtainable is not clear, for sap is expressed much more easily from toluene-treated than from frozen tissues. The reduction in dry weight as well as in sugar concentration, and possibly total solids, is presumably due to the stimulation of respiration by toluene. The diminished calcium concentration may also be associated with an increase in the rate of respiration. It is conceivable that oxalic acid is produced, which precipitates the calcium. The agreement in magnesium, potassium, and chlorine suggests that permeability is completely destroyed both by toluene and by freezing, and that as far as these solutes are concerned the composition of the expressed sap is similar to that of the sap in the intact plant.

To sum up, there are no grounds for the view that the greater concentration of chlorine in the sap than in the tissue is due to the effect of freezing. The effect of freezing on sugar concentration cannot be assessed, but it seems a fair assumption that the difference between the frozen and tolued leaves is due to sugar lost as a result of respiration.

#### (D) *Bound Water.*

The differences between the concentrations of sugar and chlorine in the whole tissue, in the sap, and in the pressed residue would appear to be real and not due to methods or technique. Their only explanation is that

TABLE V.

*Composition of Sap Expressed from Frozen and Toluene-treated Leaves (gm. per 100 gm. Water). Experiment 2, October 12, 1933.*

Treatment.	Total solids.	Calcium.	Magnesium.	Potassium.	Chlorine.	Total sugars.	Gm. water in sap expressed from 100 gm. fresh weight.
Freezing at $-5^{\circ}\text{C.}$ { 4 hrs.	7.50	0.425	0.078	0.489	0.159	1.22	57.6
24 " "	7.24	0.402	0.078	0.502	0.159	1.20	60.4
Mean	7.37	0.414	0.078	0.496	0.159	1.21	59.0
Freezing at $-15^{\circ}\text{C.}$ { 4 hrs.	7.98	0.436	0.082	0.496	0.159	1.35	59.0
24 " "	7.68	0.420	0.079	0.515	0.169	1.33	60.4
Mean	7.83	0.428	0.081	0.506	0.164	1.34	59.7
Toluene vapour	7.32	0.405	0.083	0.519	0.161	1.07	51.8
Significant { $P = 0.05$	0.39	0.020	0.006	0.061	0.013	0.18	0.530
differences { $P = 0.10$	0.31	0.016	0.005	0.047	0.010	0.14	0.415

TABLE VI.

*Composition of Sap Expressed from Frozen and Toluene-treated Corollas (gm. per 100 gm. Water). Experiment 5, October 31, 1933.*

Treatment.	Total solids.	Calcium.	Magnesium.	Potassium.	Chlorine.	Total sugars.	Gm. water in sap expressed from 100 gm. fresh weight.
Frozen at $-15^{\circ}\text{C.}$	5.54	0.0161	0.028	0.294	0.091	2.86	62.0
Frozen with $\text{CO}_2$	5.53	0.0168	0.027	0.291	0.089	2.88	62.4
Toluene vapour	5.25	0.0135	0.024	0.277	0.093	2.60	54.3
Significant { $P = 0.05$	0.32	0.0023	0.006	0.027	0.006	0.009	0.366
differences { $P = 0.10$	0.24	0.0017	0.005	0.020	0.004	0.007	0.270

a portion of the water in the tissue does not function as a solvent, and is what physiologists call *Bound Water* (4, 16). It would perhaps be more accurate to say that a larger proportion of the water in the tissue residue than in the expressed sap appears to be bound. This bound water is liberated on drying, and therefore the *concentration* estimates involving water values calculated from the loss in weight in tissue on drying are low to an extent depending on the proportion of bound (non-solvent) water to free (solvent) water in the tissue.

#### IV. BOUND WATER.

##### (A) *Estimation.*

The data presented in Table II not only demonstrate the existence of bound water, but allow calculation to be made of the extent of such bound water. In Experiment 1, 100 gm. of water in the whole tissue contained 0.519 gm. chlorine. This amount of chlorine would have been present in 90.1 gm. of sap water. Hence 9.9 gm. in every 100 gm. of water in the whole tissue cannot have been acting as a solvent for chlorine. The bound-water value, calculated from the whole tissue and sap, for this experiment is therefore 9.9 gm. per 100 gm. total water, or 37.7 gm. per 100 gm. dry matter. The bound-water value can also be calculated from the concentrations in the sap and in the pressed residue provided that the distribution of water between the expressed sap and the pressed residue is known. Thus, in Experiment 1, 100 gm. of water in the residue contained 0.315 gm. chlorine. This was equivalent to 54.6 gm. of sap water, and so 45.4 gm. out of each 100 gm. of water in the residue must have been bound. The residue contained only 24.05 per cent. of the water originally present in the tissue, the rest being expressed as sap. Each 100 gm. of water originally present in the tissue would, therefore, have contained  $45.4 \times 0.2405$ , or 10.9 gm. bound water. If the high bound-water value calculated for the residue has any basis in fact, the cytoplasm or structural elements of the leaf or both must have contained nearly as much bound as free water.

Table VII shows the amount of bound water per 100 gm. total water, and per 100 gm. dry weight for Experiments 1 to 4, calculated from sugar and chlorine data for whole tissue and sap, and for sap and residue. The values given in this table do not include bound water that might occur in the expressed sap. We have, however, failed to detect, by Newton and Gortner's (13) method, using half molecular solutions of sucrose and sodium chloride, any bound water in sap expressed from frozen tissue. The ideal method of determining sap concentrations would be one which took into account the possible existence of bound water in the sap. The estimation of chlorine concentrations by means of a chlorine electrode would appear

to be such a method, but unfortunately it has not yet proved possible in practice.

TABLE VII.

*Bound Water Calculated from Sugar and Chlorine Concentrations.*

Experiment.	Material.	Bound water from			
		Whole tissue and sap		Sap and residue	
		per 100 gm. Dry weight.	per 100 gm. Total water.	per 100 gm. Dry weight.	per 100 gm. Total water.
1	Chlorine	37.7	9.9	41.5	10.9
	Chlorine	47.5	14.6	16.3	5.0
2	Total sugars	63.1	19.4	—	—
	Sucrose	63.8	19.6	—	—
3	Chlorine	29.9	9.2	8.1	2.5
	Total sugars	46.4	14.3	—	—
4	Chlorine	31.6	11.9	19.2	7.2
	Total sugars	36.4	13.7	—	—
	Sucrose	27.4	10.3	—	—

Comparing the sugar and chlorine results for the 'whole tissue and sap' it will be seen that the sugar estimates are on the average somewhat greater than those for chlorine. As we shall have occasion to refer to this difference in a later section, we will at present merely stress the fact that both the sugar and chlorine results indicate the presence of bound water. The chlorine results calculated from the sap and the residue are, except in one instance, less than those calculated from the whole tissue and the sap. This discrepancy would arise if there were a tendency for the residue to lose moisture and/or for the estimation of chlorine in the sap to be high relative to that on oven-dried material.

We propose to avoid the difficulty of obtaining comparable analyses of sap and tissue by attempting to estimate bound water by a differential sap method. If tissue is allowed to dry out slowly there will be an increase in the sap concentration of chlorine and a decrease in the total water content. In the presence of bound water, the relative increase in sap concentration should be greater than the relative decrease in total water, the degree of divergence being a measure of bound water present.

(B) *Application to Estimation of Sap-soluble Materials.*

In order to determine what proportion of the various mineral elements are in solution in the cell sap, a knowledge of the bound-water content of the tissue would seem to be required. Methods based on the leaching of dried material or extraction by hot water are objectionable in that, apart from the release of adsorbed solutes on heating, there is a danger of chemical transformation. In the present section we shall, therefore,

illustrate the use of the method in the calculation of the proportion of some of the mineral elements that are in solution in the sap.

TABLE VIII.

*Concentrations (gm. per 100 gm. Water) in Whole Tissue, Expressed Sap, and Pressed Residue. Experiment 4, March 5, 1934.*

	Total sugars.	Sucrose.	Calcium.	Magnesium.	Potassium.	Chlorine.
Whole tissue	1.57	0.61	1.172	0.088	1.034	0.208
Sap	1.82	0.68	0.819	0.067	0.902	0.236
Residue	—	—	2.252	0.152	1.363	0.173

The concentrations of sugars and of chlorine in Experiment 4 have already been recorded, but are presented again in Table VIII for comparison with the concentrations of calcium, magnesium, and potassium. It will be seen that in contrast to the former the concentrations of these elements are greater in the whole tissue than in the sap, and are also greater in the pressed residue than in the sap or the whole tissue. If the assumption is made that all the chlorine is in true solution (no adsorption) and that the differences in concentration, to which we have just referred, are due to the presence of bound water, it is possible to calculate the solubilities of calcium, magnesium, and potassium. Making these assumptions, we find that calcium is 61.5 per cent., magnesium 67.2 per cent., and potassium 76.8 per cent. soluble. If these assumptions are justified, the view sometimes expressed that potassium is completely in solution will have to be revised (cf., however, 6). We have repeatedly found that potassium can be completely extracted from fresh leaf tissue by hot water or by leaching dried material, and this suggests that potassium not in solution is probably adsorbed.

#### (C) *Adsorption of Solutes.*

The probability of adsorbed potassium suggests that other solutes may exist in the adsorbed state. Bull (1) has pointed out that adsorption of solute may cause errors in the determination of bound water, and has shown that either zero or negative bound-water values may result. Bound-water values for sugar and for chlorine for cotton leaves are recorded in Table VII. The differences are small, and though the sugar values are on the whole greater than those for chlorine, the differences may not be significant. Differences in the adsorption of chlorine and sugar are not therefore indicated. As the cotton leaf does not appear to be a promising object for the investigation of the adsorption of solutes, an examination was made of the leaves of a number of other plants.

The names of the plants are shown in Table IX. The leaves of these plants were treated in exactly the same way as that already described for

cotton, but no attempt was made to ascertain the bound-water content of the expressed sap. The values for the bound-water content of the tissue have been calculated from the concentrations in the expressed sap and in the whole tissue. It will be seen that, in the case of cotton, the sugar values are again in excess of those for chlorine. In other words, the concentration in the sap relative to that in the tissue is higher for sugar than for chlorine. There are, of course, a variety of possible reasons that might, altogether apart from adsorption of chlorine, bring about this effect. There might, for instance, be reducing substances present that are more soluble in the sap than in alcohol. There is also the possibility of hydrolysis of polysaccharides during sap expression.

TABLE IX.

*Bound Water, as a Percentage of Total Water, Calculated from Total Sugar and Chlorine Concentrations in Sap and Tissue.*

No.		Sample.	Bound water from	
			Total sugars.	Chlorine.
1	<i>Gossypium barbadense</i> L.	<i>a</i>	23·3	17·6
		<i>b</i>	25·2	14·9
2	<i>Citrus aurantium</i> L.	<i>a</i>	13·3	33·3
		<i>b</i>	9·8	36·7
3	<i>Mammea americana</i> L.	<i>a</i>	20·0	47·1
		<i>b</i>	8·5	47·1
4	<i>Terminalia Catappa</i> L.	<i>a</i>	10·7	20·5
		<i>b</i>	13·4	15·2
5	<i>Cana</i> spp.	<i>a</i>	10·7	15·1
		<i>b</i>	15·7	20·2
6	<i>Cocos nucifera</i> L.	<i>a</i>	-17·8	1·0
		<i>b</i>	-20·6	1·6
7	<i>Cyperus rotundus</i> L.	<i>a</i>	-9·1	2·8
		<i>b</i>	-14·2	4·5
8	<i>Saccharum officinarum</i> L.	<i>a</i>	-26·7	8·0
		<i>b</i>	-51·4	0·0

In plants numbered 2, 3, 4, and 5 in Table IX the chlorine values exceed those for sugar. This might occur as a result of sugar adsorption, or the presence of sugar complexes that are not broken up in the sap but are by alcohol. It might also arise as a result of differences in the solubility of reducing substances in sap and alcohol respectively. In the plants numbered 6, 7, and 8 the sugar values are actually negative, while the chlorine values are positive. It is noteworthy that these three plants have parallel venation.

As the difference in the bound-water values for chlorine and sugar in the leaf of the sugar-cane (*S. officinarum* L.) was so great, it seemed to us probable that factors other than those just alluded to might be at work.



It was accordingly decided to carry out a more detailed examination on this plant. The most probable reason for the large difference appeared to us to be that the sap expressed might not be a true sample of the sap in the intact leaf. It will be evident that, if there are marked regional (e.g. vein and mesophyll) differences in the distribution of sugar and chlorine in the cane leaf, and/or if all the tissues that compose the leaf are not rendered completely permeable to both these solutes by the temperature ( $-15^{\circ}\text{C}.$ ) employed, anomalous results would ensue.

Certain modifications of procedure were therefore introduced. The cane leaves were rapidly minced and frozen with carbon dioxide for about sixteen hours. On withdrawing from the 'freezing tubes', the tissue was allowed to thaw for about twenty minutes in a covered beaker. Three sub-samples were then drawn, one for sap expression, a second for moisture determination, and a third for alcohol extraction. Chlorine was determined on the alcohol extract and also on the dried material. Two samples, one taken shortly after the other, were used and each was done in duplicate. The determinations of sucrose and reducing sugars were carried out both on cleared and uncleared solutions.

The results are shown in Table X. It will be seen that the bound-water values for chlorine and for sucrose are in good agreement in both samples and with both cleared and uncleared solutions. Reducing substances show larger values than chlorine and sucrose on the cleared solutions and smaller on the uncleared.

TABLE X.

*Concentrations (gm. per 100 gm. water) in Sap and Tissue, and Bound Water Calculated from these Concentrations.*

<i>Sample A.</i>					
Solutes.	Cleared.		Uncleared.		Chlorine.
	Reducing sugars.	Sucrose.	Reducing sugars.	Sucrose.	
Tissue . . . .	0.49	1.85	0.61	1.84	0.194
Sap . . . . .	0.56	1.98	0.62	2.01	0.209
Bound water as a per cent. of total water }	12.5	6.6	1.6	8.5	7.2
<i>Sample B.</i>					
Tissue . . . . .	0.41	2.09	0.51	2.09	0.168
Sap . . . . .	0.47	2.30	0.54	2.32	0.186
Bound water as a per cent. of total water }	12.8	9.1	5.6	9.9	9.7

As reducing substances are chemically heterogeneous, it seems unlikely that this fraction, at least in the leaf (cf. 15), can be used for the determination of bound water. To sum up, the agreement between the bound-water values for sucrose and for chlorine suggests that neither is adsorbed, for it

is unlikely that they would be adsorbed to the same extent. For potassium, on the other hand, there are grounds, as we have already pointed out, for the belief that a considerable proportion is not in solution in the sap.

#### V. SUMMARY.

1. Estimates of the weight of chlorine in the leaf of the cotton plant, calculated from the concentration in the expressed sap and the weight of water in the leaf, exceed by more than 10 per cent. estimates based on the weight of chlorine in the oven-dried material. Similarly, the sap estimates of the weight of sugar in the leaf exceed estimates based on alcoholic extraction.

2. Likewise, the concentrations of chlorine and sugar in the *sap* exceed the concentrations in the whole tissue, the latter being calculated from the weights of chlorine and sugar in the whole tissue and the weight of water found by drying the tissue to constant weight at 100° C.

3. As there is no indication of the presence of bound water in the expressed sap, using the cryoscopic method, it is suggested that the reason for the discrepancy in the sap and tissue estimates is due to the presence of bound water in the tissue.

4. This suggestion is reinforced by the observation that the concentration of chlorine in the residue left after sap expression is much less than in the expressed sap. The concentration of sugar in the residue after pressing is vitiated as a result of hydrolysis of polysaccharides.

5. It is pointed out that the *weight* of sap-soluble potassium, and probably the weight of many other sap solutes, cannot be determined by leaching the powdered oven-dried material, or by hot water extraction, owing to the presence of adsorbed potassium, &c., which are released by heating. It is calculated that about 25 per cent. of the total potassium is not in solution in the cell sap.

6. It is also emphasized that the *concentrations* of sugar and chlorine in the intact plant are best obtained on sap expressed from frozen tissue; possibly the concentrations of the solutes could also be obtained by correcting the tissue concentrations for bound water by means of the calorimeter or dilatometer.

7. No evidence of the adsorption of sucrose or chlorine has been obtained.

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# Further Studies on Transport in the Cotton Plant.

## V. Oxygen Supply and the Activation of Diffusion.<sup>1</sup>

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With twenty Figures in the Text.

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<sup>1</sup> Paper No. 15 from the Physiological Department of the Cotton Research Station, Trinidad.  
[*Annals of Botany*, Vol. L. No. CXCI. July, 1936.]

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## I. INTRODUCTION.

THE problem presented by the very rapid rate at which solutes travel through the sieve-tube system is one of the most perplexing that confronts the plant physiologist. Mason and Maskell (20) calculated that the rate of carbohydrate transport is from twenty to thirty-eight thousand times as rapid as physical diffusion. Two general types of mechanism have been suggested in order to account for the rapid rate at which materials travel through the sieve-tube. The first involves movement through the vacuole and the second through the cytoplasm. The first has been most ably expounded by Münch (25). The essential feature of his *Druckstrom-hypothese* is that the sieve-tube does not play an active part in the movement of materials through it. Solution is driven under pressure through the vacuole and the sieve-pores which are assumed to be open. Energy is supplied in the leaf by the metabolic activity of the transition cells (27), which accumulate solutes from the mesophyll, and maintain the turgor pressure gradient in the sieve-tube system. Thus, as long as the turgor pressure gradient is maintained and the sieve pores remain open, transport should continue. The *Diffusion Theory* contemplates movement through stationary cytoplasm (cf. Huber (12), Mason, Maskell, and Phillis (22)) and through sieve-pores filled with cytoplasm. Diffusion is activated in some way by the energy released in the respiration of the sieve-tube (23). Solutes travel independently of one another and of water; and the rate and direction of movement of each solute are determined by its own gradient.

Our knowledge of the respective parts played by cytoplasm and vacuole in this movement is still rather fragmentary. Knowledge of the part of the sieve-tube in which movement occurs would help to elucidate the mechanism, while information concerning the mechanism would help to define the path. Structural considerations do not suggest that solution can travel longitudinally through the vacuole and the sieve-pore. In the first place, the sieve-pores are filled with a solid core of cytoplasm

(Schmidt (30), Crafts (3, 4), and Schumacher (32)). Even if this core of cytoplasm were readily permeable to solutes, the resistance opposed to the *Druckstrom* would still be very great. Other characters that do not harmonize with a mass flow of solution are the presence of sieve-tubes with imperfect sieve-plates at the bundle ends (Fischer (10)) and the existence of compact balls of parenchymatous cells separating the sieve-tubes of neighbouring internodes in many of the Dioscoreaceae (Mason (18)). Further, as the vacuolar sap may contain a highly viscous slime and is often very granular, movement of the contents of the vacuole from one sieve-tube to another would rapidly block the sieve-pores.

Schumacher (32) has attempted to determine directly in what part of the sieve-tube transport actually occurs by means of microscopic observations using the fluorescent dye fluorescein. When blobs of gelatine containing a dilute solution of the dye were placed on the scraped dorsal surface of the veins, he found that the dye moved into the bundle, where first the companion cell and later the sieve-tube were stained. After a short time, the dye spread downwards through the sieve-tube into the petiole *without a trace being visible in the vacuole*.<sup>1</sup> He concluded that movement occurred in the cytoplasm of the sieve-tube. While this may have been the case, the possibility does not seem to be wholly excluded that the dye travelled back in the wood, spread outwards through the rays, and after concentration by the companion cell was released into the sieve-tube. We have found, for instance, that in the cotton plant the dye may travel back in the wood and enter a sieve-tube below a ring. We have not yet succeeded in demonstrating the presence of the dye in the sieve-tube in flaps of bark not in contact with the wood.

Among observations designed to throw light on the mechanism of transport and which might, therefore, indirectly help to define the path, we may mention the work of Curtis and Scofield (6) on the osmotic pressures of supplying and receiving organs, Weevers and Westenberg's (35) work on the exudation of water from flaps of bark prised away from the wood, and the attempt of Mason, Maskell, and Phillis (22) to make sugar and nitrogen travel simultaneously in opposite directions through the phloem. Curtis and Scofield's work is not crucial largely because companion cells might accumulate solutes even from flaccid cells and thus generate high concentrations in the sieve-tube. Weevers and Westenberg failed to demonstrate that transport was active in the flaps of bark employed, though probably it was, as cambial growth occurred in controls. Mason, Maskell, and Phillis succeeded in demonstrating that sugar might travel down the stem at the same time that nitrogen was travelling upwards from the old leaves, but they failed with their system of rings to

<sup>1</sup> According to Döring (9) the distribution of the dye in the cell depends on the pH (cf. also Keller (14)).

rule out the possibility that nitrogen travelled down the phloem to the root, where it was released into the wood and travelled upwards with the transpiration current. Very recently, however, Phillis and Mason (28) have succeeded in demonstrating the simultaneous movement of carbohydrate and nitrogen in reverse directions in the phloem, thereby considerably strengthening the grounds for a diffusion basis for transport.

Observations have from time to time been made with a view to determining whether transport depends on the activity of living cells. In carrying out such experiments, it is essential that the concentrations in the supplying and receiving regions should not be directly affected by the treatment of the intervening channel. Secondary changes in concentration due to a block in the channel are, of course, inevitable. Unfortunately, much of the work done in this direction is not beyond criticism. Starch has often been used as the only criterion. Where dry weights have been used, it has generally been assumed that the changes are due to carbohydrate, and that photosynthesis and respiration in the leaf are unaffected by the treatment accorded the channel. The basis of expression has generally been the fresh weight or the area; in no instance do the results appear to have been expressed on the sample basis. Moreover, no estimate has been obtainable of the *extent* to which carbohydrate transport was checked, nor does the transport of mineral elements in the phloem appear to have been considered.

Czapek (7) killed petioles by heat and by chloroform and found that export from the lamina was checked. As the cytoplasm in the pores must have been coagulated, transport may have been checked for this reason and not because cytoplasm plays an active part in movement. With dilute chloroform jacketing the petiole, transport was also checked, but plasmolysis of the sieve-tube apparently did not check movement. Kruseman (15) also found that mild narcotics checked transport. Curtis (5) found that chilling the petiole from 25° C. to 7° C. did not greatly influence transport, but that chilling between 1° C. and 5° C. markedly checked movement. Kruseman obtained conflicting results on the effect of chilling on transport. Summing up, it would appear that mild narcotics check movement, and that the effect of temperature cannot be explained in terms of viscosity changes. The data, however, seem incomplete and further work with narcotics and temperature appears desirable.

Finally, we come to the question of oxygen supply and transport. Wortman (36) found that coating the petioles of *Pelargonium* leaves with wax delayed the removal of starch from the lamina. Curtis (5) found that the carbohydrate content of red kidney bean leaves did not appear to be appreciably affected by waxing the petiole, and thought that this was due to aeration through the petiolar cavity. On the other hand, he showed that enclosing the petioles in tubes containing nitrogen under slight

pressure led to an increase in the carbohydrate content of the lamina, and concluded from this that transport had been checked. In view of the great importance of the relation between oxygen supply and transport, and of the necessity for a rigid experimental demonstration of the relation (cf. 26), we decided to further investigate the problem. If oxygen supply is a factor in movement and respiration releases energy for the acceleration of diffusion, the *Druckstromhypothese* of Münch would become untenable; and, since streaming of the protoplasm of the mature sieve-tube does not occur (Huber (12)), some other method of accelerating diffusion would be indicated. In this connexion, it is important to distinguish between the entry of solutes into the phloem at the bundle ends and longitudinal transport through the sieve-tubes. It would seem, whatever the mechanism of longitudinal transport through the sieve-tube may be, that work must be done and oxygen consumed in the process of solute accumulation by the transition cell (cf. 27).

## II. THE LEAF.

Two experiments will be described in this section. In both, the whole surface of the petiole was covered with a mixture of four parts of paraffin wax and one part of vaseline. Observations were then made on the effect of this coat on the dry weight and the mineral content of the leaf. Observations were also made on the rate of protoplasmic streaming in the phloem parenchyma of the petiole.

### (A) *Experiment 1.*

#### (1) *Procedure.*

The plants were fourteen weeks old and had been maintained in a vegetative condition by removal of the flower buds. Four mature leaves on each plant were tagged, and the petioles of two were coated with wax. The tagged leaves were situated on the sixth to the ninth nodes from the apex on the main axis. The rest of the leaves, amounting to about 90 per cent. of the total number, were removed before the petioles were waxed. At each collection, two samples, each of twenty-five leaves, were taken. The application of warm wax and vaseline might, it was thought, cause injury to the underlying tissues. Before applying the wax, the petioles were therefore coated with 5 per cent. gelatine. This was allowed to dry in the sun and then coated with wax. The sequence of events was as follows:

#### *Time-table.*

Feb. 6.	1933.	All except four tagged leaves stripped from plants.
		Petioles of Wax group coated with gelatine and wax.
„ 7.	„	1st collection of Normal and Wax Leaves (8 a.m.).

H h



Feb. 7.	1933.	2nd collection of Normal and Wax leaves (3 p.m.).
" 8.	"	3rd collection of Normal and Wax leaves.
" 9.	"	4th collection of Normal and Wax leaves.
" 10.	"	5th collection of Normal and Wax leaves.
" 11.	"	6th collection of Normal and Wax leaves.
" 13.	"	7th collection of Normal and Wax leaves.
" 14.	"	8th collection of Normal and Wax leaves.

After collection, the petioles were separated from the laminae. Three petioles from each sample were taken at random and used for making observations on the rate of protoplasmic streaming, the cut ends being dipped into melted wax. The remaining petioles were cleaned by wiping first with a dry cloth to remove the wax, then with a wet cloth to remove the gelatine, and finally with a dry cloth. They were then weighed and sampled for dry weight determination and for freezing for sap expression. The laminae were weighed and sampled for dry weight and for sap expression. The results are expressed on the sample basis and represent the weights of material present in twenty-five laminae or petioles as the case may be.

For the examination of protoplasmic streaming in the phloem parenchyma, free hand sections were cut under liquid paraffin<sup>1</sup> and mounted in the same medium. Five observations on the rate of streaming were made on each of the three petioles withdrawn from each sample. The total time of procedure of mounting and observation did not exceed ten minutes. This time limit was imposed to lessen the possibility of recovery of streaming due to oxygen leakage. We have found that when streaming is halted by placing the petiole in nitrogen, recovery, when it does occur, generally exceeds ten minutes.

## (2) *Results.*

(a) *Carbohydrate.* The dry weights of the lamina of the Normal and of the Wax groups are shown on the right of Fig. 1.<sup>2</sup> It will be seen that initially the Wax group exceeded the Normal. The rapidity of the response, within twenty hours of waxing, suggests that transport was checked very soon after the application of the wax. After the third collection, however, the Normal exceeded the Wax group. It may be significant that at this period the concentration of sugar in the lamina of the Wax group was greatly in excess of that in the Normal (see Fig. 3). Between the 5th and 8th collections the rates of dry weight accretion were about

<sup>1</sup> Previously deoxygenated by heating in vacuo.

<sup>2</sup> Significant differences are shown in this and in succeeding figures for two levels of significance. The outer (longer) pair of lines are for the level  $P = 0.05$  (full significance), and the inner (shorter) pair for  $P = 0.10$  (partial significance).

the same in both groups; both transport through the petiole and carbon assimilation by the lamina may have been checked by the wax. In the petiole (Fig. 1, left) the dry weights of the Wax group throughout exceeded those of the Normal. The difference between the two groups increased

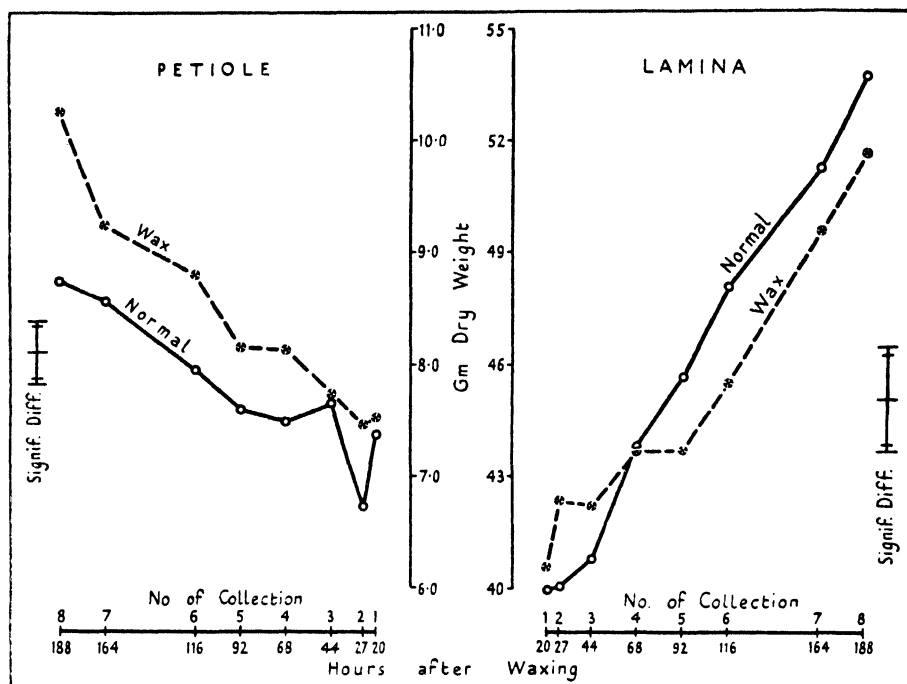


FIG. 1. Dry Weights in Lamina (Right) and Petiole (Left) for Normal and Wax Groups with Significant Differences for  $P = 0.05$  and  $P = 0.10$  (Experiment 1).

steadily throughout the experiment. The results suggest that transport through the petiole was checked and that the rate of carbon assimilation was reduced between the 2nd and 5th collections as a result of waxing the petiole.

Interest centres chiefly on the earlier collections, when the dry weights of the lamina of the Wax group exceeded those of the Normal. The differences between the dry weights of the lamina of the two groups were not fully significant at any one of the first three collections, but the difference at the 2nd collection and the *mean difference* for the three collections (see Table I) reach the level of partial ( $P = 0.10$ ) significance. These differences would appear to be due to differences in labile carbohydrate,<sup>1</sup> the values for which are shown in Fig. 2. It will be seen (Table I) that the mean carbohydrate difference exceeded that for the dry weight.

<sup>1</sup> Total sugars after inversion plus acid hydrolysable polysaccharides.

TABLE I.

*Gm. Labile Carbohydrate and Dry Weight in Normal and Wax Groups at First Three Collections.*

Collection.	Labile carbohydrate.			Dry weight.		
	Normal.	Wax.	Wax minus Normal.	Normal.	Wax.	Wax minus Normal.
1	3.95	5.92	1.97	39.99	40.55	0.56
2	5.82	7.77	1.95	40.03	42.36	2.33
3	3.44	6.39	2.95	40.81	42.20	1.39
Mean			2.29			1.43
Signif. Diff. { $P = 0.05$			0.32			1.60
of Mean Diff. { $P = 0.10$			0.25			1.27

The concentrations of total sugars (after inversion) in the sap present certain features of interest. It will be seen (Fig. 3) that in the Normal group the concentration is greater in the lamina than in the petiole. This is the first occasion (cf. 27) on which we have found the sugar concentration in the lamina greater than in the petiole. The unusually low concentration in the petiole is probably due to carbohydrate starvation in the body of the plant as a result of the preliminary stripping of the leaves. In the Wax group, the concentrations are of the same order in lamina and petiole. Comparing the concentrations in the two groups, it will be observed that Wax exceeded the Normal in both lamina and petiole, and that the differences are much more marked in the petiole than in the lamina. The results are indicative of a block in the channel of transport in the petiole.

(b) *Phosphorus and calcium.* The results for phosphorus and calcium are shown in Fig. 4. The graphs show the changes in the weights of these elements in lamina and petiole for the two treatments. In the petiole, calcium which is relatively immobile in the phloem, shows an important difference in behaviour to the highly mobile element phosphorus. No statistical data are presented for the petiole, as the two samples were bulked for purposes of chemical analysis. The weight of phosphorus in the Wax group exceeded that in the Normal, while the reverse obtained for calcium. As the weights of both elements were greater in the Normal than in the Wax group in the lamina, the results are evidently what would obtain if transport through the petiole and transpiration from the lamina were checked as a result of waxing the petiole.

(c) *Protoplasmic streaming.* The effect of waxing on protoplasmic streaming in the phloem parenchyma of the petiole is shown in Fig. 5. The significant difference is large, but, as far as can be judged, there was no marked effect of waxing on streaming. As streaming stops after some hours in nitrogen, it may be concluded that oxygen was not completely cut off from the petiole. The results suggest that the oxygen requirement for

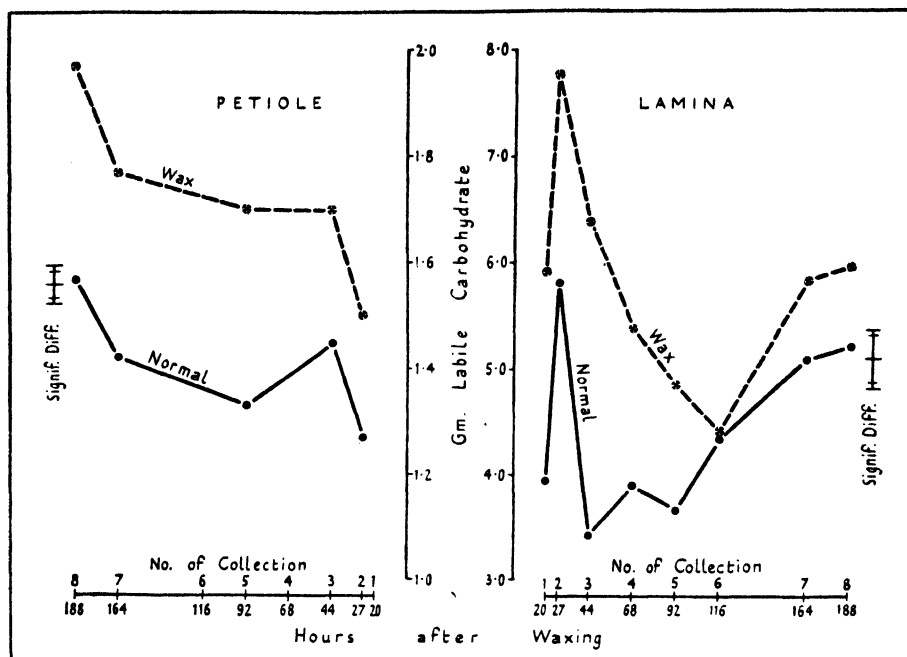


FIG. 2. Weights of Labile Carbohydrate in Lamina (Right) and Petiole (Left) for Normal and Wax Groups (Experiment 1).

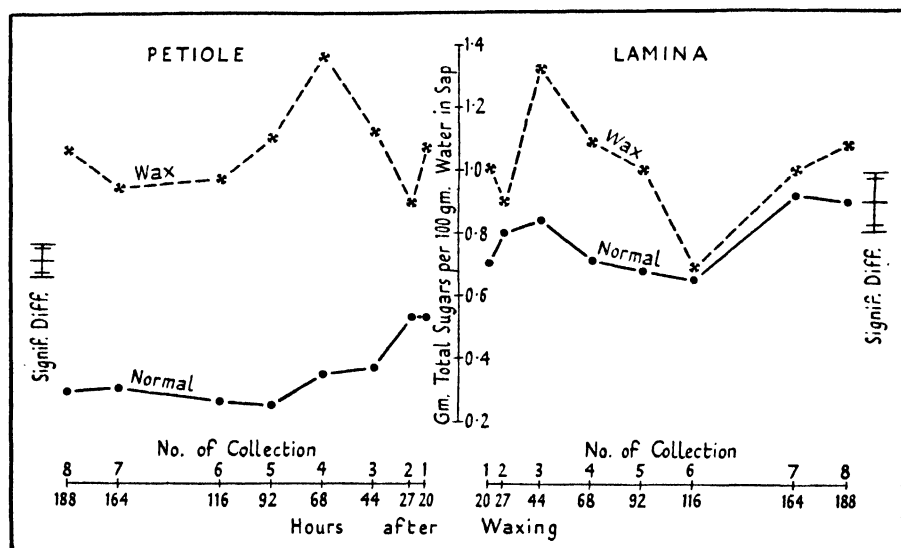


FIG. 3. Concentrations (gm. per 100 gm. Water) of Total Sugars in Sap of Lamina (Right) and Petiole (Left) for Normal and Wax Groups (Experiment 1).

normal transport may be greater than that for protoplasmic streaming and *substantiate the conclusion that protoplasmic streaming is not a factor accelerating diffusion.* They are also important, in that they indicate that any disturbances that may have arisen as a result of partial anaerobic respiration in the petiole were so small that protoplasmic streaming remained unaffected. It seems unlikely, consequently, that the products of anaerobic respiration could have been responsible for the apparent check in transport. It would also seem unlikely that high temperatures in the petiole beneath the wax could have affected transport. In this connexion we have found that the disappearance of starch is delayed from laminae whose petioles are coated with wax even when the whole plant is placed in the dark.

(B) *Experiment 2.*

(1) *Procedure.*

This experiment differed from that just described in two important respects. Firstly, the plants were not defoliated. There was thus competition for water and solutes between the leaves sampled and the rest of the leaves on the plant. The leaves with wax-coated petioles were thus, in this experiment, competing against a much larger number of Normal leaves than in the first experiment. Secondly, of the three leaves sampled on each plant one was left unwaxed and two were waxed. Of the two waxed, the wax was removed from one after five days. This was done to determine what injury had been done, directly or indirectly, by the application of the wax. There were thus three treatments in this experiment, namely, Normal, Wax, and Remove. The procedure was otherwise similar to that of the first experiment. Data, however, are available only for the lamina, as the petioles were not sampled. There were two samples per collection and fifty leaves per sample. The results are again expressed on the sample basis.

*Time-table.*

Jan. 23.	1933.	Waxing of petioles.
„ 24.	„	1st collection of Normal and Wax leaves.
„ 25.	„	2nd collection of Normal and Wax leaves.
„ 27.	„	3rd collection of Normal and Wax leaves.
„ 28.	„	4th collection of Normal and Wax leaves.
„ „	„	Removal of wax from petioles of Remove group.
„ 30.	„	5th collection of Normal, Wax, and Remove leaves.
„ 31.	„	6th collection of Normal, Wax, and Remove leaves.
Feb. 2.	„	7th collection of Normal, Wax, and Remove leaves.
„ 3.	„	8th collection of Normal, Wax, and Remove leaves.

(2) Results.

(a) *Carbohydrate*. The results for total carbohydrate<sup>1</sup> in the lamina are shown in Fig. 6. Initially, as in Experiment 1, the lamina of the Wax group shows an accumulation of carbohydrate relative to the Normal group.

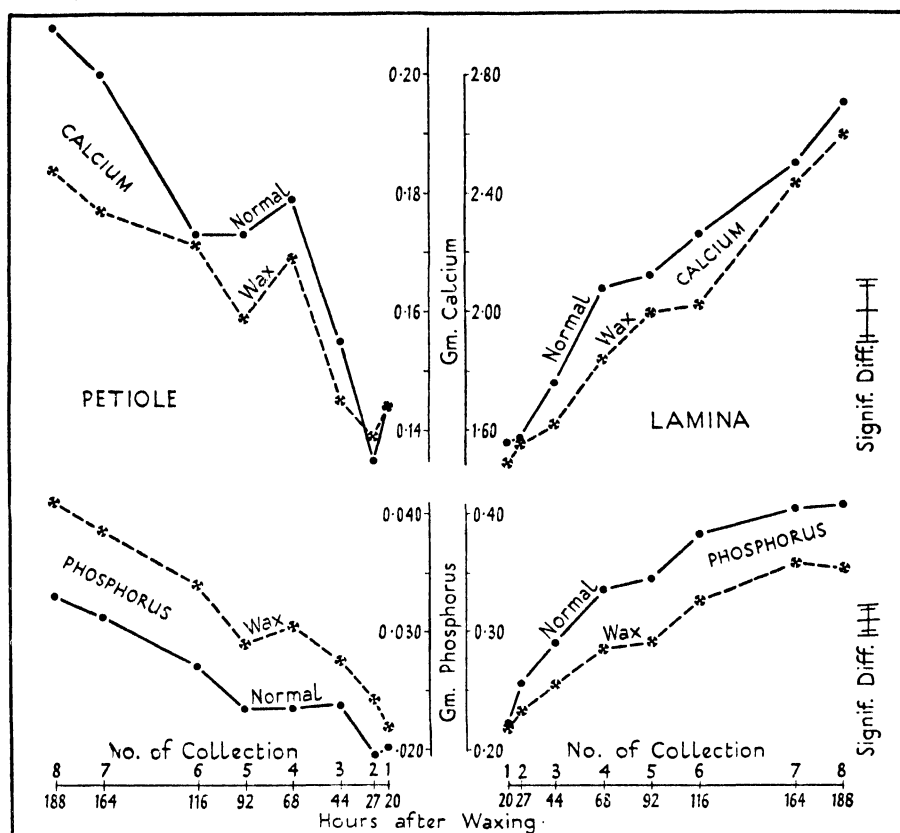


FIG. 4. Weights of Phosphorus (Lower) and Calcium (Upper) in Lamina (Right) and Petiole (Left) for Normal and Wax Groups (Experiment 1).

The rapidity of response is again noteworthy, the interval between applying the wax and the 1st collection being less than twenty-four hours. After the 2nd collection, the Wax group falls below the Normal, but, unlike Experiment 1, there is a net loss of carbohydrate from the Wax group. On removal of the wax from the petioles, recovery is rapid, the rate of accretion being if anything greater than in the Normal group.

(b) *Mineral elements*. The results for six mineral elements are shown in Fig. 7. The correlation coefficients for the Normal and Wax groups

<sup>1</sup> In this experiment total carbohydrate represents the dry weight from which the weight of mineral elements (viz. 5.7 nitrogen + 3.05 phosphorus) is deducted.

between the weights of the various elements in the lamina and time are recorded in Table II. Fully significant losses of nitrogen, phosphorus, and potassium are shown by the Wax group. Chlorine and magnesium also show negative coefficients in this group, but the coefficients are not significant. Calcium, on the other hand, shows a positive correlation coefficient with time which is fully significant. With the exception of nitrogen, which shows a small but insignificant negative correlation, all elements show positive correlations in the Normal group. It will be clear that transport of the mineral elements was not stopped by waxing the petiole. The losses are considerable (e.g. nitrogen 40 per cent., potassium 32 per cent.) and do not suggest that transport was even checked, though nothing is known concerning the rates of entry of the mineral elements into the lamina.

TABLE II.

*Correlation Coefficients<sup>1</sup> Between the Weights of Mineral Elements and Time in Normal and Wax Groups.*

Group.	Nitrogen.	Phosphorus.	Chlorine.	Potassium.	Magnesium.	Calcium.
Normal	-0.386	+0.955	+0.760	+0.660	+0.893	+0.918
Wax	-0.979	-0.723	-0.524	-0.975	-0.348	+0.740

It will be recollected that waxing the petiole led to an initial accumulation of carbohydrate. For two elements, potassium and chlorine, the values (cf. Fig. 7) for the Wax group are initially in excess of those of the Normal group, but the differences do not attain the level of partial significance.

Removal of the wax from the petiole was followed either by no further loss (nitrogen and magnesium) or by actual gains (phosphorus, chlorine, and potassium). In the case of calcium alone, removal of the wax was without effect. It is striking that for phosphorus, chlorine, and potassium the gains by the Remove group exceeded those of the Normal. As these elements entered the leaf in the transpiration current, it would appear that recovery in transpiration may have preceded recovery in transport.

### (C) *Discussion.*

It will be convenient to discuss Experiments 1 and 2 together. The first effect of coating the petiole with wax is an accumulation of carbohydrate in the lamina in excess of the normal amount. This is followed in a few days by a diminution in the amount of carbohydrate below that in the lamina of the untreated leaf. In this latter stage there is also a reduction in the mineral content of the leaf. It would appear that both

<sup>1</sup> Fully significant ( $P=0.05$ ) correlations are shown in heavy type, and partially significant ( $P=0.10$ ) correlations in italics.

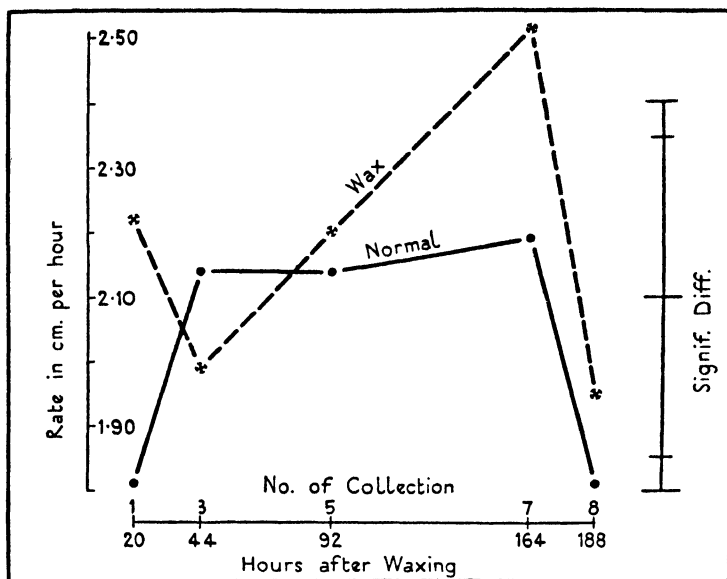


FIG. 5. Rates of Protoplasmic Streaming in Phloem Parenchyma of Petioles for Normal and Wax Groups (Experiment 1).

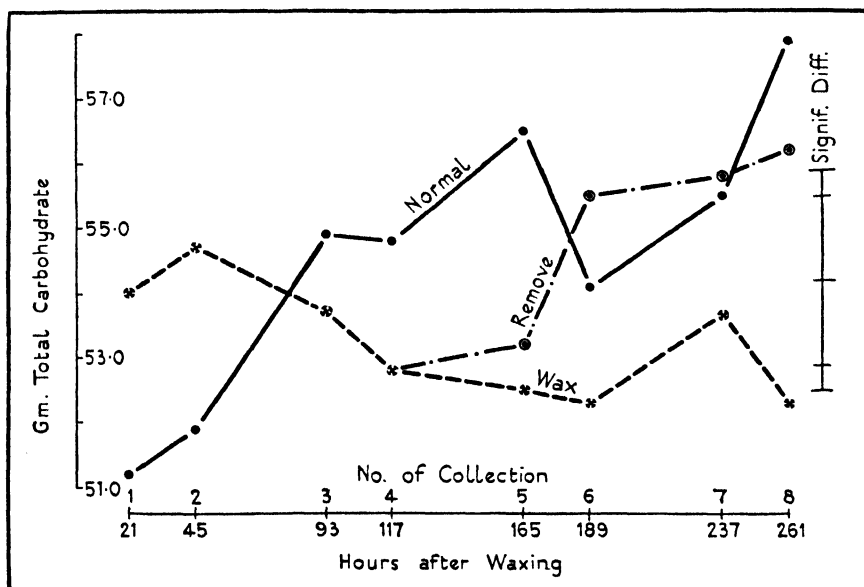


FIG. 6. Weights of Total Carbohydrate for Normal, Wax, and Remove Groups (Experiment 2).



photosynthesis and transpiration receive a check. This might arise in a number of ways, among which may be mentioned partial closure of stomata as a result of the accumulation of sugar and/or the accumulation in the lamina

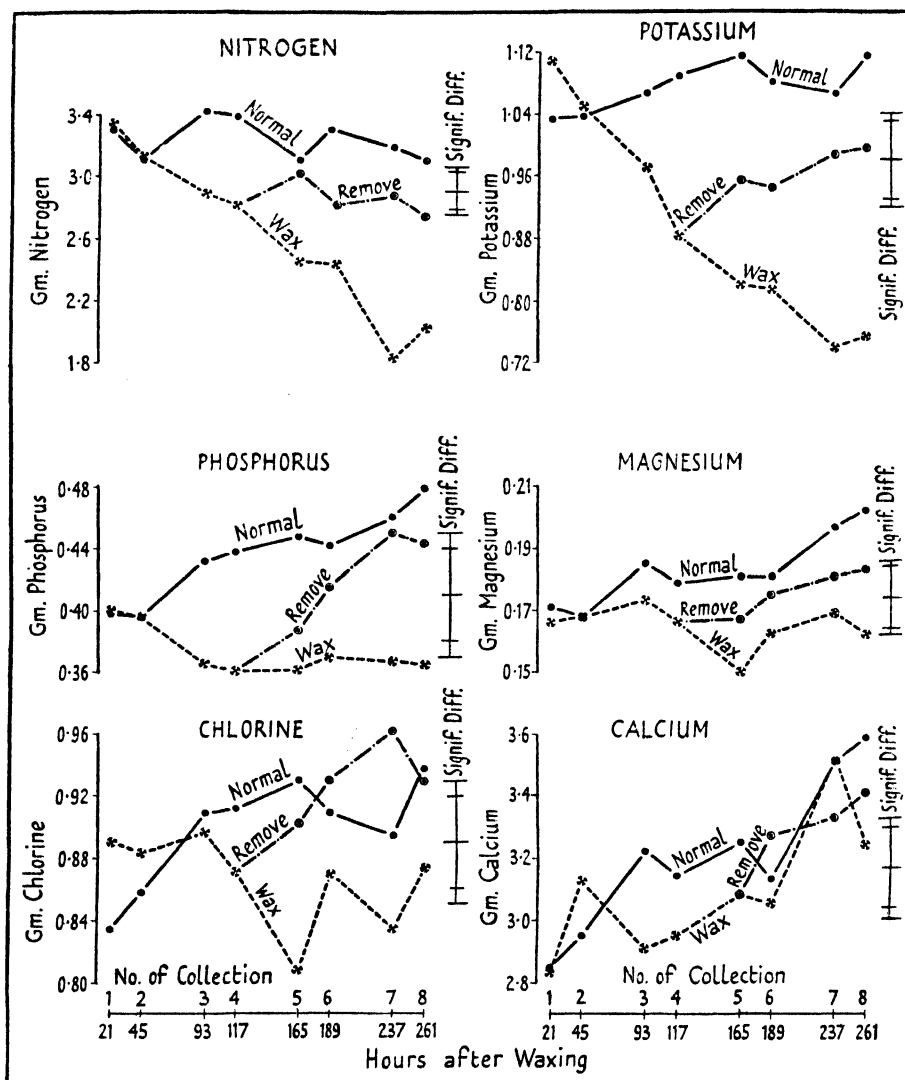


FIG. 7. Weights of Various Mineral Elements for Normal, Wax, and Remove Groups (Experiment 2).

of the products of anaerobic respiration in the petiole. A reduced supply of mineral elements might further diminish the rate of protosynthesis. It might also affect the utilization of mineral elements and the general course of metabolism of the lamina.

It is, however, with the initial accumulation of carbohydrate in the lamina, which occurred in both experiments, and the piling up of carbohydrate and phosphorus in the petiole, which was observed in Experiment 1, with which we are concerned. These results are, of course, indicative of an obstruction in the channel of transport. The behaviour of the mineral elements in Experiment 2 gives pause, however, to this interpretation. The exodus of nitrogen and potassium from the lamina was so marked, that one is forced to inquire whether there may not be another explanation of the initial carbohydrate accumulation in the lamina. That we are dealing with different channels for the transport of carbohydrate and the mineral elements would seem untenable (cf. Schumacher (31)).

It seems clear, that if no check in transport occurred as a result of the coat of wax on the petiole, the cause of the above phenomena must be sought in the lamina. It is conceivable that the application of wax to the petiole led to an initial stimulation of photosynthesis or to a reduced rate of respiration. If this took place as a result of the arrival in the lamina of the products of anaerobic respiration, anaerobic respiration must have set in with great rapidity. Moreover, neither an increase in the rate of photosynthesis nor a reduction in the rate of respiration in the Wax lamina would account for the piling up of phosphorus in the petiole and its exodus from the lamina. The behaviour of phosphorus might, however, be accounted for if the breakdown of phosphorus compounds in the lamina was for any reason accelerated by waxing the petiole.

On the whole, it seems more likely that the accumulation of carbohydrate in (Experiments 1 and 2) and loss of mineral elements from (Experiment 2) the lamina, as well as the piling up of carbohydrate and phosphorus in the petiole (Experiment 1), were due to a *partial* block in the channel of transport in the petiole combined with a check in the rate of transpiration. However, even if it be conceded that there was a partial block in transport in the petiole, it does not follow that respiration is responsible for the activation of diffusion in the sieve-tube, for the check in transport might have arisen as a result of toxic compounds produced in anaerobic respiration. This, as we have suggested, is unlikely in view of the continuation of protoplasmic streaming in the wax-coated petioles. It would appear that this type of experiment, in which only the 'source' is sampled, and in which the rate of carbohydrate production and of uptake of the mineral elements by the leaf are unknown, is ill-adapted to determining the role, if any, of oxygen in transport.

### III. THE STEM.

In the previous section it was shown that coating the petiole with wax led to inconclusive results. While the results obtained may have been due to a partial block in the channel of transport, they give no estimate of how

complete the block may have been, though the great losses of nitrogen, phosphorus, and potassium from the lamina in Experiment 2 suggest that

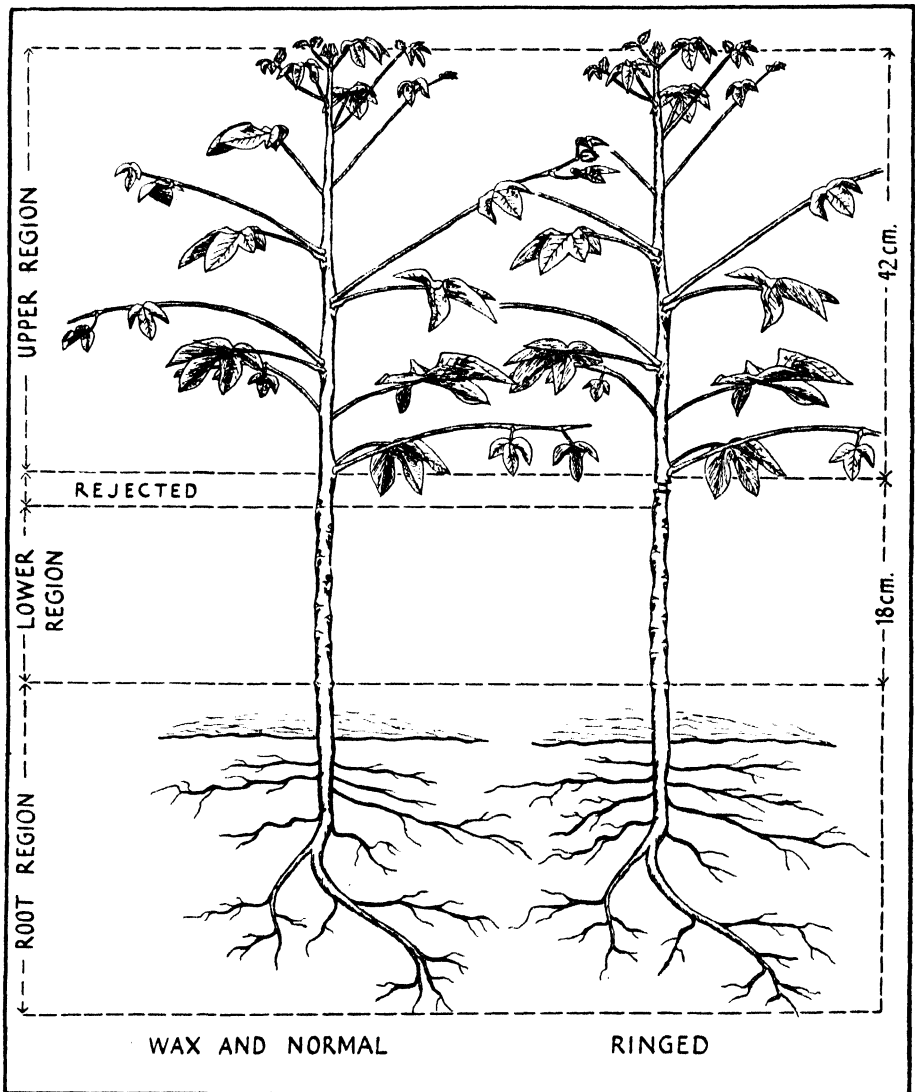


FIG. 8. Diagram Showing Treatment of Plants for Normal, Wax, and Ringed Groups (Experiment 3).

it cannot have been very marked. On the other hand, the accumulation of carbohydrate and phosphorus in the petiole in Experiment 1 was considerable. With a view to ascertaining whether curtailing the air supply causes a check in transport, and *to what extent*, we have attempted to

exclude air from the stem, utilizing the root in one experiment and a portion of the stem in another as the 'sink'. No information concerning the effect of oxygen supply on the transport of mineral elements can, of course, be expected from this type of experiment owing to uncertainty concerning uptake by the stem from the transpiration current. In the second experiment rubber bands were substituted for wax, as the latter appeared as if it might be rather porous, and estimates of the alcohol content of the bark beneath the rubber were made.

(A) *Experiment 3.*

(1) *Procedure.*

The mean height of the plants was 60 cm. The stem was divided (see Fig. 8) into two regions, an Upper region with foliage leaves and a Lower region of bare stem between the foliage region and the root. Upper and Lower regions were divided in such a way that each contained the same number of nodes. There were three treatments—Normal, Ringed, and Wax. The Ringed plants were ringed immediately below the Upper region. The internode with the ring in the Ringed group and the corresponding internode in the other groups were rejected, and are not included in either the Upper or the Lower region. The stem of the Lower region of the Wax group was coated with wax. The length coated with wax was approximately 18 cm. The ringing and waxing operations were done, and an initial collection of 'tops' made on March 21, 1933. The plants, Upper, Lower, and Root regions, were collected, and the dry weights determined on March 31. There were two samples, each of twenty-five plants, per treatment. The results are as usual expressed on the sample basis.

(2) *Results.*

The dry weights of the Whole Plant are shown in Table III, the Root, of course, being included in the Whole Plant. It will be seen that both ringing and waxing may have had a slightly depressing effect on dry weight accretion. During the ten days of the experiment the 'tops' of the Normal group increased in dry weight by 85 per cent. No initial dry weight was obtained for the Root, so that the percentage increase for the Root is not known. To allow for differences in dry weight accretion and/or respiration, we have expressed the regions below the ring, that is the Lower region and the Root, as percentages of the Whole Plant. It will be seen that the sink, that is the Root, was in the Normal group 17.93 per cent., in the Ringed group, with transport stopped, 10.27 per cent., and in the Wax group 16.03 per cent. of the Whole Plant. In the stem beneath the wax the difference between the Normal and the Wax group was even more marked.

TABLE III.

*Dry Weight of Whole Plant and Dry Weights of Lower Region and of Root as Percentages of Whole Plant in Normal, Wax, and Ringed Groups.*

	Whole plant.		Lower region as percentages of whole plant.		Root	
Normal <i>a</i>	1992.7	} 2056.00	9.18	} 9.38	18.73	} 17.93
" <i>b</i>	2119.3		9.58		17.14	
Wax <i>a</i>	1941.7	} 1966.55	7.29	} 7.11	16.61	} 16.03
" <i>b</i>	1991.4		6.93		15.46	
Ringed <i>a</i>	1851.9	} 1932.80	6.96	} 6.68	10.18	} 10.27
" <i>b</i>	2013.7		6.41		10.36	

### (3) Discussion.

The results show that waxing the stem for approximately 18 cm. did not stop, but only checked transport into the root over a period of ten days. There are difficulties in the way of assessing the exact extent to which transport was checked, but if we take the value 10.27 per cent., the relative dry weight of the Root of the Ringed group, as representing no transport, and deduct this value from the corresponding values for the Normal and Wax groups, we obtain values of 7.66 for the Normal and 5.76 for the Wax groups. Transport would therefore appear to have been checked by the wax to the extent of about 25 per cent. Whether oxygen was supplied longitudinally via the wood or phloem, or horizontally by leakage through the wax, is not clear. Nor is it certain that the reduction in transport was not due to the products of anaerobic respiration. It would appear that the growth of the stem beneath the wax was depressed to a greater extent than was transport through it.

### (B) Experiment 4.

#### (1) Procedure.

The plants used in this experiment were twenty-two weeks old, and were as usual maintained in a vegetative condition by removal of the flower buds. As in the last experiment, there was (see Fig. 9) an Upper region with foliage and a Lower region of bare stem. The latter was subdivided into a Top and a Bottom region, each with an equal number of nodes. The plants were subdivided into regions on the basis of number of nodes, and were graded on the length of the Bottom region. There were nine plants per sample and four samples per collection. There were three treatments (see Fig. 9), all of which were ringed below the Bottom region. This was the only treatment accorded the Normal group. In the Ringed group, rings were also made on the internodes separating the Upper and Top regions and the Top and Bottom regions. These three internodes were rejected and are not included in the dry weights. The stems were trimmed in the laboratory on the stipular marks, as shown in Fig. 9. In the

Rubber and Ringed groups, cycle-tyre inner tubes, cut longitudinally, were banded around the whole length of the Top region. The length of the stem banded with rubber was approximately 54 cm. The rubber was coated with rubber varnish. The root was not sampled, the sink for

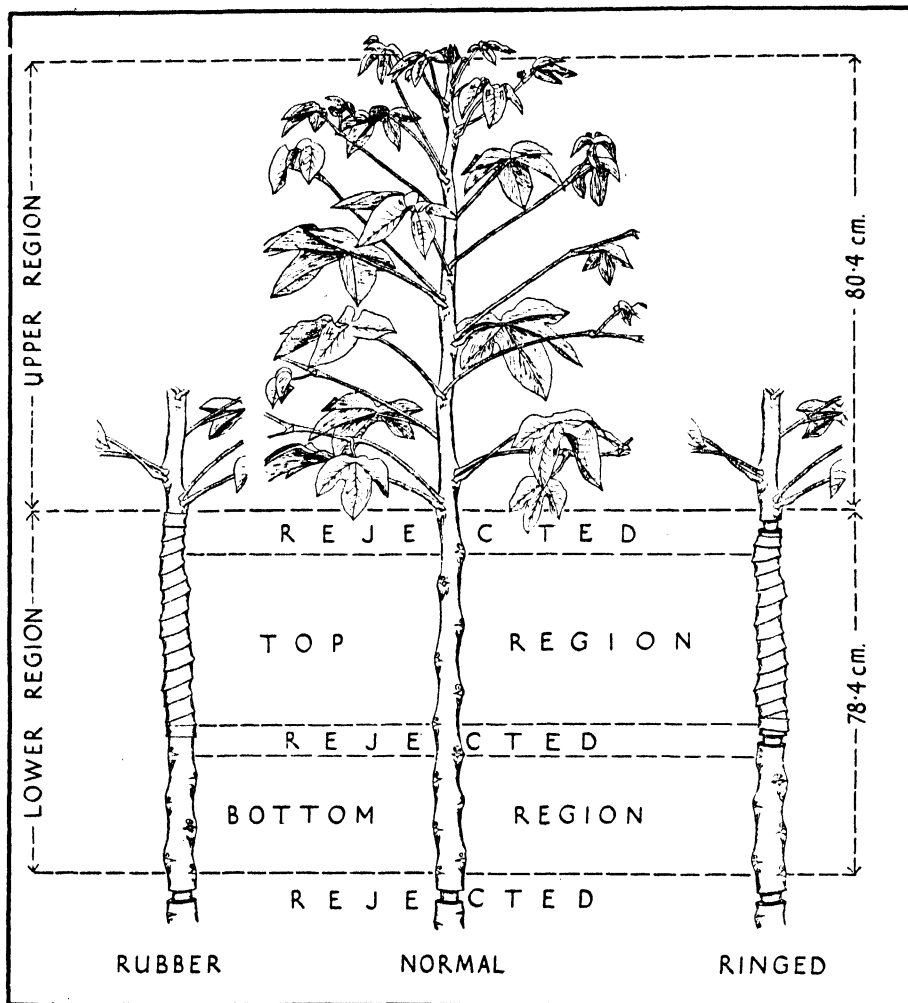


FIG. 9. Diagram Showing Treatment of Lower Region of Plants for Normal, Rubber, and Ringed Groups (Experiment 4).

registering transport in this experiment being the Bottom region. Estimates of the alcohol<sup>1</sup> content of the Top bark were made on three samples

<sup>1</sup> Samples of approximately 50 gm. were dropped into 350 ml. of water at 75° C. and then slowly distilled for two hours, the distillate being collected in water at 0° C. The 200–250 ml. of distillate collected was then redistilled, the first 100 ml. being collected, and from this second distillate samples were taken for the estimation of alcohol by oxidation with alkaline permanganate (34). Substances other than alcohol are, of course, included by this procedure.

at each collection. For comparison with the three treatments already described, six samples of Top stem, covered with rubber just as in the Rubber and Ringed groups, were cut off the plants and removed from the field. This group is referred to as the Isolated Rubber group. After waxing the exposed ends these stems were placed in a box in the laboratory. Alcohol determinations were made on the bark of three samples of this group on the days of the 2nd and of the 3rd collections. The results for dry weight are expressed on the sample basis and for alcohol per 100 gm. fresh weight.

*Time-table.*

Jan. 18, 1934.	All plants ringed below Bottom region.
" 19, "	Ringling of Ringed group and banding of Rubber and Ringed groups (7-11 a.m.).
" " "	Initial collection of Normal group (12 noon).
" 22, "	Removal to Laboratory of Isolated Rubber group.
" 23, "	1st collection of Normal, Rubber, and Ringed groups.
" 29, "	2nd collection of Normal, Rubber, and Ringed groups and 1st collection of Isolated Rubber group.
Feb. 9, "	3rd collection of Normal, Rubber, and Ringed groups and 2nd collection of Isolated Rubber group.

(2) *Results.*

The dry weights for the *Whole Plant above the basal ring* (i.e. Upper, Top, and Bottom regions), which we shall refer to as the Whole Plant, are shown on the right of Fig. 10. It will be seen that there is but little difference between the Normal and Ringed groups, but that the dry weight of the Rubber group is quite definitely down on these two groups. Consideration of the dry weights of the Bottom region alone without reference to the dry weight of the Whole Plant would, therefore, lead to erroneous conclusions concerning the rates of carbohydrate transport down the stem. As in the last experiment, we therefore express the dry weights of the sink, in this case, the Bottom region, as percentages of the dry weights of the Whole Plant. These results are shown graphically in Fig. 10 (lower left). If we take the differences between the Ringed and the other two groups as indices of the rate of transport, we find no evidence that banding the Top stem with rubber has affected the rate of transport. The dry weights of the stem beneath the rubber, that is, the Top region, expressed as percentages of the Whole Plant, are shown at the upper left of Fig. 10. It will be seen that the rubber has had a markedly depressing effect on the dry weight of the stem beneath it.

The dry weights of the *bark* of the Top region are shown in Fig. 11. The results are again expressed as percentages of the dry weight of the Whole

Plant. It will be seen that the dry weight in the Normal group increased slightly throughout the experiment, while in the Ringed and Rubber groups there were small declines, the decline in the Ringed group exceeding that in the Rubber group. The concentrations of total sugars in the bark

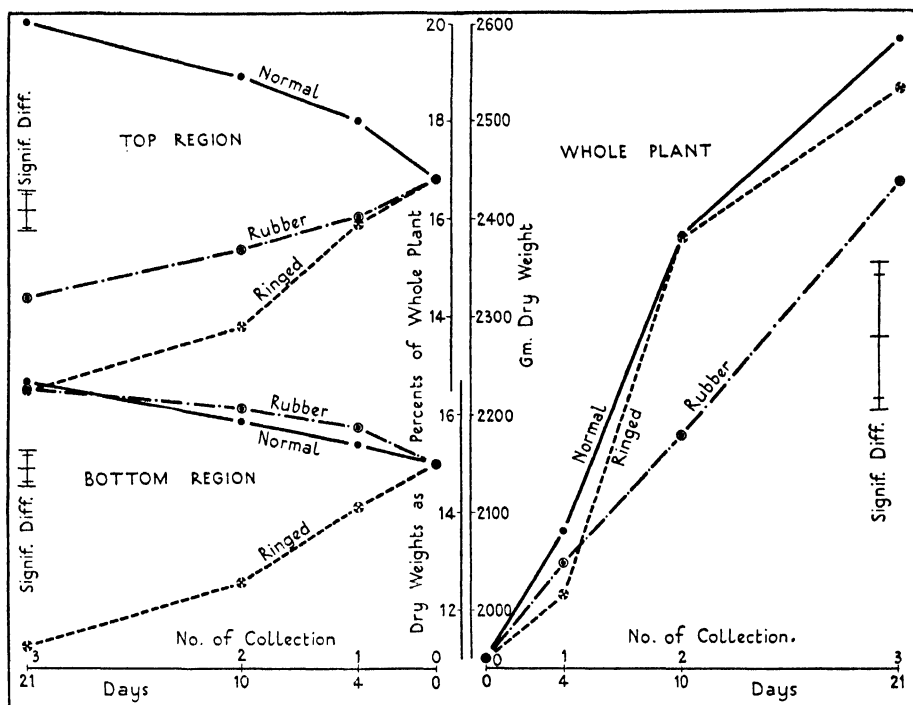


FIG. 10. Dry Weights of Whole Plant above Basal Ring (Right), and Dry Weights of Bottom Region (Lower Left) and Top Region (Upper Left) as Percentages of Whole Plant, for Normal, Rubber, and Ringed Groups (Experiment 4).

of the Top region are shown in Fig. 12. It will be seen that the sugars have accumulated in the Rubber group in excess of the Normal, the mean concentrations (gm. per 100 gm. water) being Normal 3.55, Rubber 4.20, and Ringed 2.57. The utilization of sugar, or possibly its conversion into starch, has been affected by banding the stem.

The weights of alcohol per 100 gm. fresh weight in the bark of the Top region are shown in Fig. 13. It will be noticed that there has been an enormous accumulation of alcohol in the bark of the Isolated Rubber group. In the Normal group there was only a trace of alcohol, while there were small amounts in both Rubber and Ringed groups. The mean amounts per 100 gm. fresh weight and per 100 gm. water for the three collections are shown in Table IV. The Rubber group contained a significant excess over the Ringed and the Ringed over the Normal.



TABLE IV.

*Mean Weights of Alcohol Expressed in Gm. per 100 Gm. Fresh Weight and per 100 Gm. Water in Bark of Top Region of Normal, Ringed, Rubber, and Isolated Rubber Groups.*

Group.	Fresh weight.	Water.
Normal	0.010	0.016
Ringed	0.021	0.028
Rubber	0.038	0.056
Significant differences $\left\{ \begin{array}{l} P = 0.05 \\ P = 0.10 \end{array} \right.$	0.007 0.006	0.009 0.008
Isolated Rubber (mean of two collections)	0.739	0.986

### (3) Discussion.

The salient feature of this experiment is the apparent independence of transport on oxygen supply, for it seems probable that transport was unaffected while conditions of oxygen starvation prevailed beneath the rubber band. Oxygen starvation in the bark is suggested by the great production of alcohol in the bark of the Isolated Rubber group. The much smaller concentrations in the Ringed<sup>1</sup> and Rubber groups may have been due to leakage into the transpiration current. On the other hand, oxygen may have been taken up from the transpiration current in these groups and alcohol production in the bark reduced. The difference in the alcohol concentrations of the Rubber and Ringed groups may have been due to the greater sugar concentration of the former. It should be stressed that the concentration of alcohol in the Rubber group was only 0.056 gm. per 100 gm. water and that the machinery of transport was unaffected by this concentration.

A further indication of oxygen starvation is supplied by the check in the growth of the stem beneath the rubber (Rubber group). Thus, we found that the dry weight in this group was much less than in the Normal group, though greater than in the Ringed. It is remarkable that growth should be depressed, as we noted in the previous experiment, while transport may or may not be affected.

The results would suggest that respiration is not a factor in transport, were it not for the fact that in the previous experiment we noted that a check of about 25 per cent. took place as a result of waxing the stem. The results of the two experiments reported in Section II are also indicative of a partial block in the channel. The results for the experiment at present under consideration may perhaps be reconciled with those of previous experiments, if we assume that oxygen is normally supplied through the lenticels, but may, under conditions of reduced supply through the lenticels, be obtained from the transpiration current. The extent of the latter supply may well be conditioned by the rate of transpiration. The transpiration

<sup>1</sup> The Ringed group was also banded with rubber (cf. Fig. 9).

rate may, therefore, be one factor determining whether transport will be affected by the exclusion of, or reduction in, the external supply of oxygen as a result of waxing or banding with rubber.

As to why this supply of oxygen from the transpiration current is not

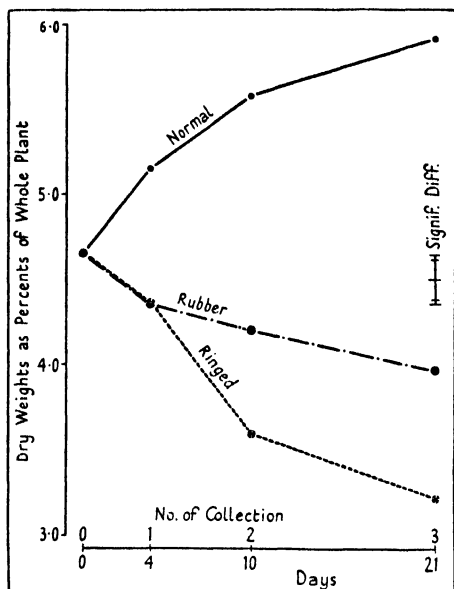


FIG. 11.

FIG. 11. Dry Weights of Bark of Top Region as Percentages of Whole Plant for Normal, Rubber, and Ringed Groups (Experiment 4).

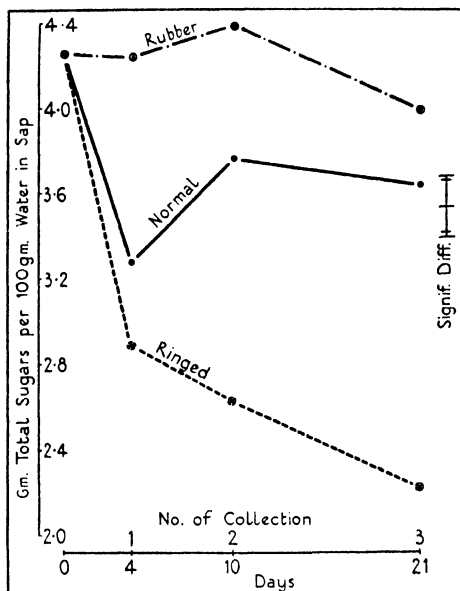


FIG. 12.

FIG. 12. Concentrations (gm. per 100 gm. water) of Total Sugars in Bark Sap for Top Region of Normal, Rubber, and Ringed Groups (Experiment 4).

available for growth, it may be suggested that the companion cells *concentrate* oxygen as well as sugar (cf. 27). Many years ago Raciborski (29) observed that with  $\alpha$ -naphthol the sieve-tubes and companion-cells gave a strong peroxidase reaction, while the surrounding cells did not. He named this peroxidase *leptomin*. We have confirmed this observation of Raciborski and have found that, though the reaction is somewhat uncertain, the sieve-tubes of plants that are frozen before the stem is cut stain intense blue. Keeble and Armstrong's (13) observations on 'bundle peroxidases' would seem to fall into line here. It may be that *leptomin* is associated with some carrier of oxygen and that the companion-cells can step up the oxygen potential, so that transport is able to proceed, while growth outside the sieve-tube is arrested. The compact nature of the phloem suggests that its oxygenation may depend on some carrier of oxygen rather than on molecular oxygen. Whether there is any longitudinal transport of oxygen through the phloem will be considered in the next section.

## IV. THE BARK.

In Experiment 3 we found that waxing the stem caused a 25 per cent. reduction in the rate of transport, and in Experiment 4 that banding the stem with rubber had no influence on the rate of movement. In both experiments, growth in the region of the covered stem seems to have been depressed, and in Experiment 4 further evidence of oxygen starvation is provided by the production of alcohol in the bark beneath the rubber. It would follow that either oxygen is not necessary for transport and that transport in Experiment 3 was checked for some other reason as a result of the application of the wax, or else that oxygen is essential, and in Experiment 4 was supplied in sufficient amounts for normal transport either through the rubber or longitudinally via the wood or bark. If the latter assumption is correct, it should be possible, provided oxygen is not supplied longitudinally through the bark and provided there is no considerable storage of oxygen, to bring about a complete cessation of transport by isolating the bark from the wood and excluding oxygen from it. In the present section, therefore, we describe two experiments, in both of which the bark was prised away from the wood for a short distance and attempts made to wholly exclude oxygen from it. In Experiment 5 the outside of the bark was heavily coated with silica varnish, while the inside, on which the varnish would not hold because of the moist surface, was coated with vaseline. In Experiment 6 we attempted to exclude oxygen by immersing the bark in mineral lubricating oil.

(A) *Experiment 5.*(1) *Procedure.*

The plants used in this experiment were divided into three regions as in the last experiment (see Fig. 14). The Top and Bottom regions were each approximately 16.5 cm. in length, the Top being the region from which oxygen was excluded, while the Bottom was used as the sink for registering transport. The plants were all ringed below the Bottom region immediately before the experiment began. There were five treatments: (1) A Ringed group in which an additional ring was made, as shown in Fig. 14. (2) A Normal group in which the bark of the Top region was prised away from the wood in three strips over a distance of approximately 14.5 cm. The three strips were tied back in place and covered with moistened sacking to prevent drying. The moistened sacking would, of course, also prevent heating of the bark by the sun. (3) A Both group in which the bark was prised away as in the Normal group, and the outside coated with silica varnish and the inside with vaseline. The bark was then tied back and kept moist as in the Normal group. (4) An In group and (5) an Out group. The plants of these groups were treated in the same

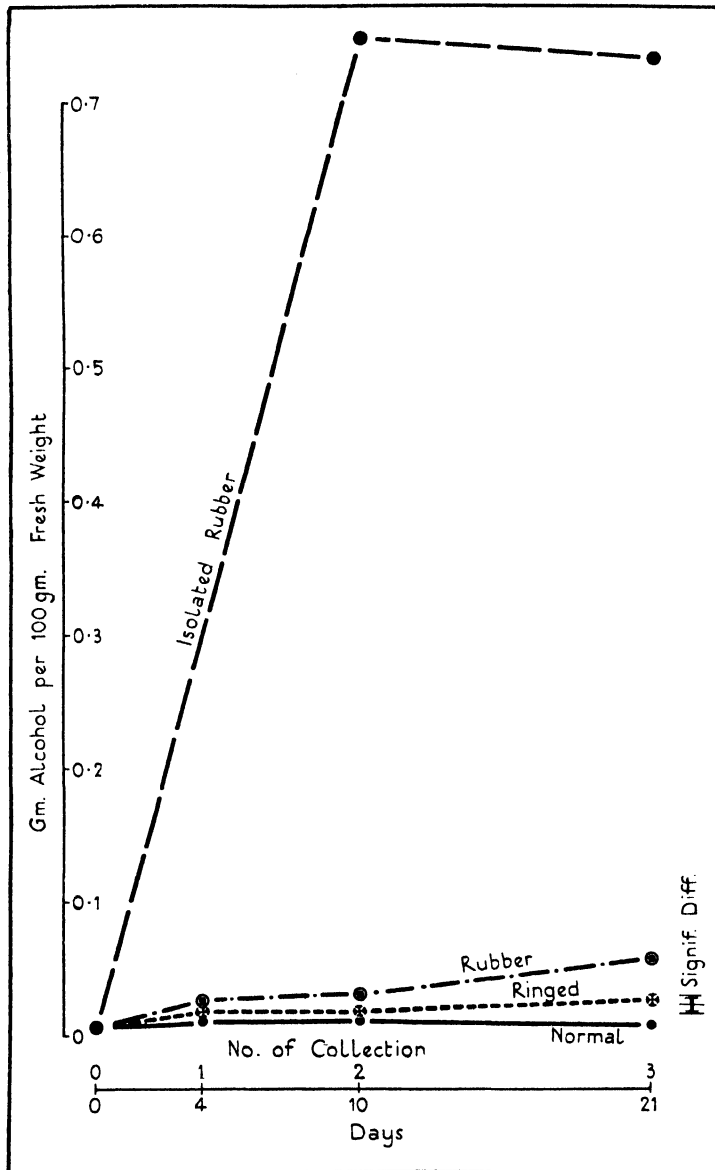


FIG. 13. Weights (gm. per 100 gm. Fresh Weight) of Alcohol in Bark of Top Region for Normal, Rubber, Ringed, and Isolated Rubber Groups, with Significant Differences for Normal, Rubber, and Ringed Groups (Experiment 4).

way as the Both group, but in the In group the inner surface of the bark was coated with vaseline and the outer surface was untreated, while in the Out group the outer surface of the bark was coated with silica varnish and the inner surface was untreated.

Grading was done on the basis of circumference of the Bottom region. There were two samples per collection and twenty plants per sample. The results are expressed on the sample basis, while alcohol is expressed per 100 gm. fresh weight.

*Time-table.*

March 21.	1934.	Ringling of all plants below Bottom region.
„ 22.	„	Separation of bark and wood, &c. in Normal, Both, In, and Out groups; also making upper ring in Ringed group (7 to 11.30 a.m.).
		Initial collection of Normal group (1 p.m.).
„ 27.	„	1st collection of Normal, Both, In, Out, and Ringed groups.
„ 29.	„	2nd collection of Normal, Both, In, Out, and Ringed groups.
„ 31.	„	3rd collection of Normal, Both, In, Out, and Ringed groups.

(2) *Results.*

The dry weights of the Whole Plant above the ring at the base of the Bottom region are shown to the left of Fig. 15. It will be seen that there are no significant differences between the five groups. The depressing effect on the dry weight of the Whole Plant which arose in Experiment 4 as a result of excluding air from a limited region of the stem is here absent. It would seem that when bark and wood are isolated, anaerobic respiration in the former no longer causes a depression in the production of dry matter.<sup>1</sup> The results for the sink, in this case the Bottom region, are therefore expressed on the sample basis and not, as in previous experiments, as percentages of the Whole Plant. The dry weights for the Bottom region are presented on the right of Fig. 15. It will be seen that the Normal and

<sup>1</sup> In Experiments 1 and 2 the application of wax to the petiole resulted in a diminution in the dry weight of the lamina and presumably in photosynthesis. It was suggested that this might have arisen as the result of the accumulation of sugar or of the products of anaerobic respiration. In Experiment 3 statistically significant differences in the dry weights of the Whole Plant between the treatments are absent, but both the Ringed and Wax groups appear to have had smaller dry weights than the Normal group. In Experiment 4 all treatments had a basal ring, and the dry weight of the Whole Plant of the Rubber group was definitely less than those of the Ringed and Normal groups. As the Ringed group was also banded with rubber, the differences between it and the Rubber group may have been due to the higher alcohol content of the latter and its liberation into the transpiration stream, &c. It may be noted that waxing the petiole leads to a partial closure of the stomata of the lamina.

In groups and that the Both and Out groups behaved alike, the latter pair being intermediate between the former pair and the Ringed group.

Alcohol was determined on the bark of the Top region. The results,

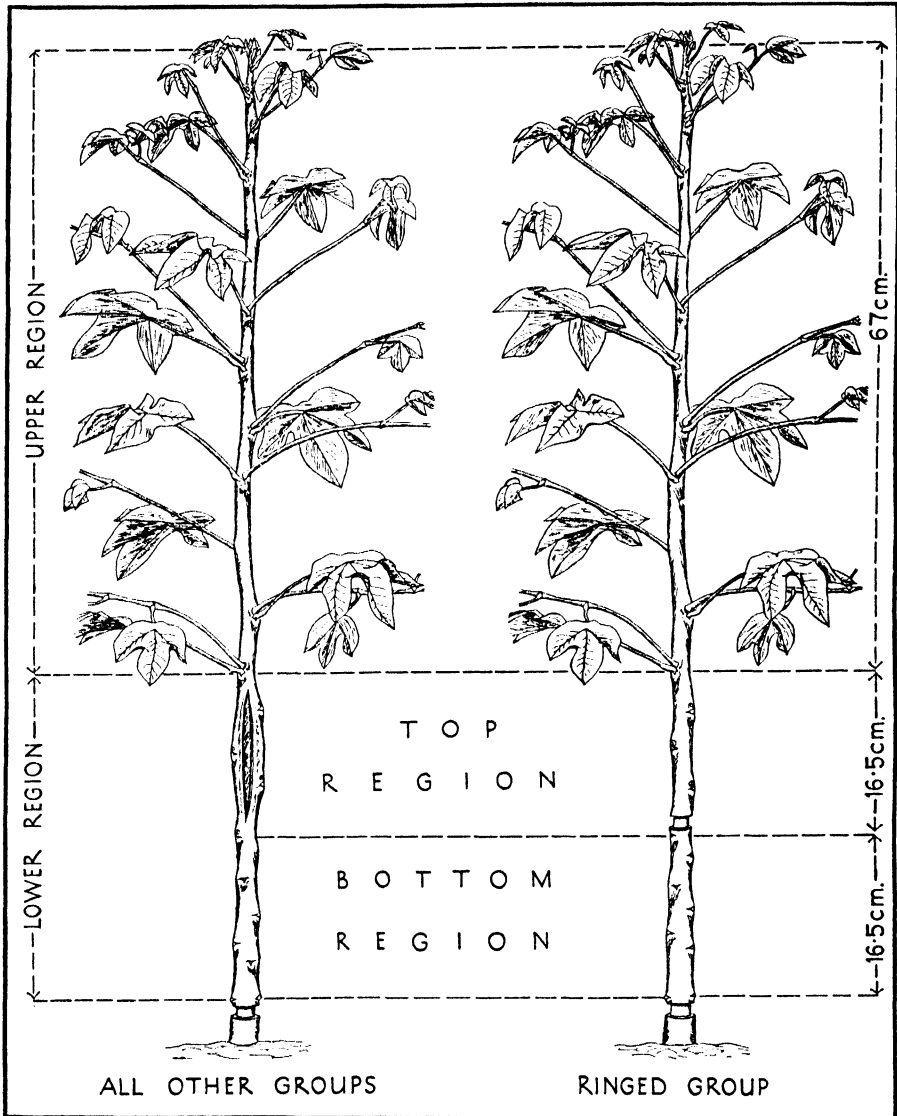


FIG. 14. Diagram Showing Treatment of Plants for Normal, Both, In, Out, and Ringed Groups (Experiment 5).

which are expressed per 100 gm. fresh weight, are shown in Fig. 16. The outstanding feature of the graph is the considerable accumulation of alcohol by the bark of the Both group. The other groups all show a much smaller

accumulation, the Normal and Ringed groups showing the smallest values, with the In and Out groups only slightly greater. Alcohol must have escaped from the Out group between the flaps or through the wood. The mean weights per 100 gm. fresh weight and per 100 gm. water are shown

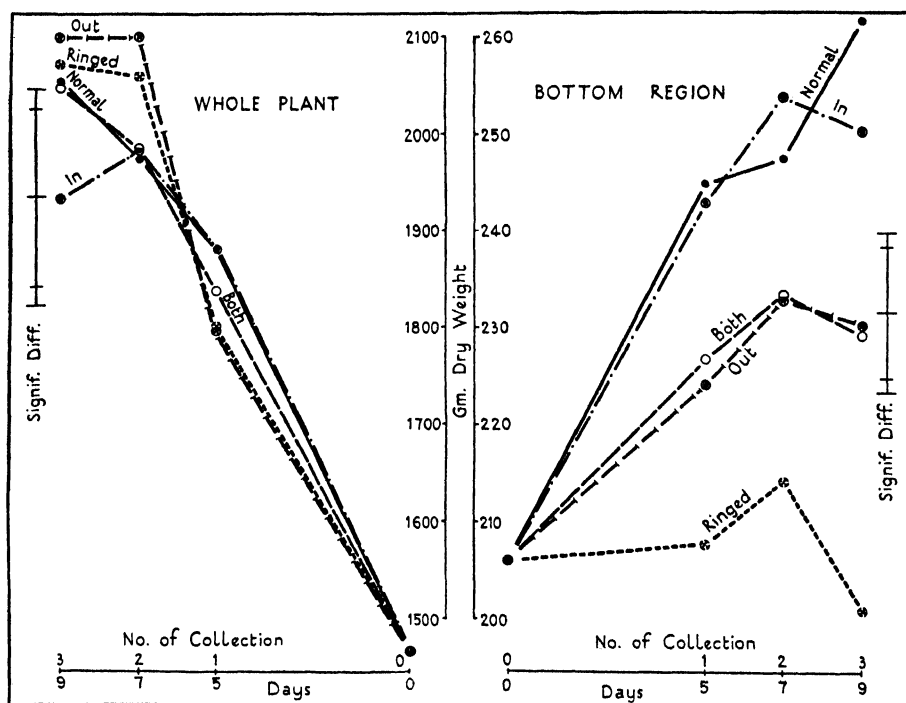


FIG. 15. Dry Weights of Whole Plant (Left) and of Bottom Region (Right) for Normal, Both, In, Out, and Ringed Groups (Experiment 5).

in Table V. It will be noticed that the mean concentration in the Both group is five times that in the Out group, while transport in both groups was checked to the same extent.

TABLE V.

Mean Weights of Alcohol Expressed in Gm. per 100 Gm. Fresh Weight and per 100 Gm. Water in Bark of Top Region of Normal, Ringed, In, Out, and Both Groups,

Group.	Fresh weight.	Water.
Normal	0.013	0.018
Ringed	0.014	0.021
In	0.019	0.026
Out	0.022	0.031
Both	0.110	0.155
Significant differences { $P = 0.05$	0.012	0.017
{ $P = 0.10$	0.010	0.014

## (3) Discussion.

The results show that, provided the bark is isolated from the wood, a coating of silica varnish on the *outside* of the bark brings about a marked reduction in the rate of transport. The application of vaseline to the *inside* of

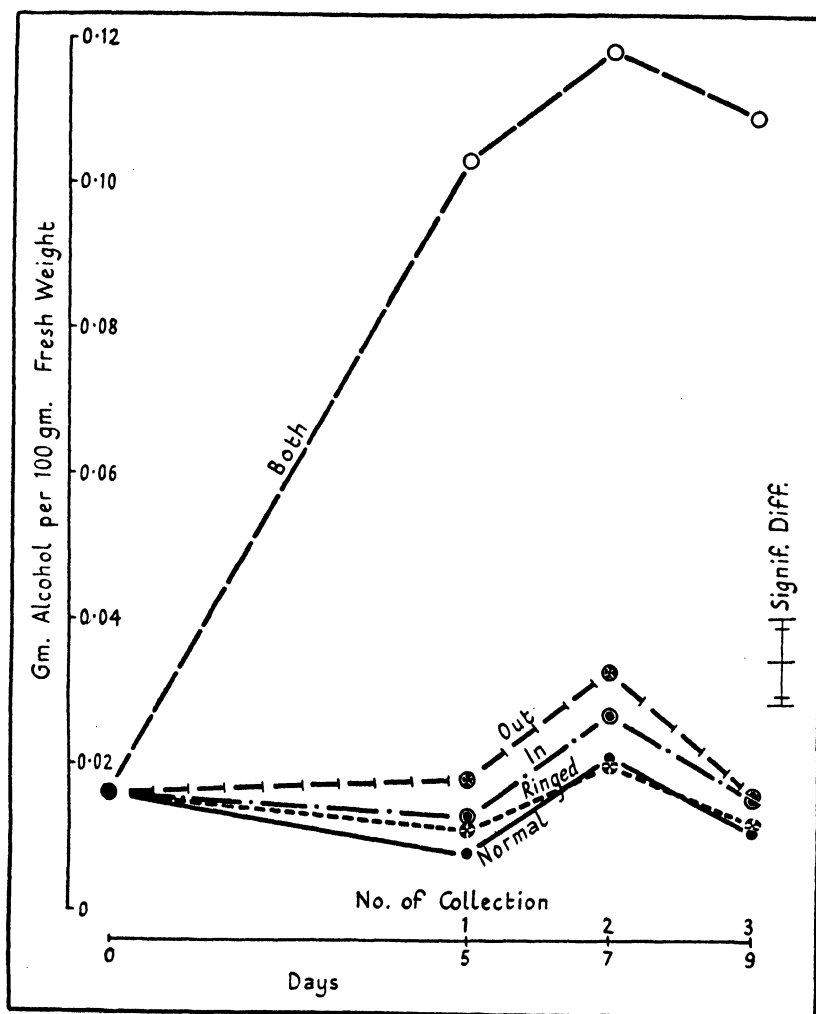


FIG. 16. Weights (Gm. per 100 Gm. Fresh Weight) of Alcohol in Bark of Top Region for Normal, Both, In, Out, and Ringed Groups (Experiment 5).

the bark was apparently without effect (cf. Normal and In groups). The actual reduction in the rate of transport calculated as in Experiment 3, and using the means of the Out and Both and of the Normal and In groups respectively, was 50.9 per cent. at collection 1, 48.0 per cent. at collection 2, and 47.9 per cent. at collection 3. The block in transport is here approximately



twice as great as in Experiment 3. The block in transport might be caused because energy is required and was not supplied by respiration, or it might be due to poisoning by the products of anaerobic respiration, of which alcohol might be taken as an example. If the latter had been the case, it would be expected that transport through the flaps would have varied inversely with the concentration of alcohol, but comparison of the Out and Both groups shows that this is not so. The mean alcohol concentration in the Both group flaps was five times that in the Out group, yet transport was checked to the same extent in the two groups. It might perhaps also be expected, had the check in transport been due to toxins, that it would have been augmented as the experiment proceeded.

It must, we think, be concluded that the partial block is due to the fact that respiration plays an integral part in transport, and that it is not due to secondary causes arising out of the accumulation of the products of anaerobic respiration. It will be recollected that in Experiment 1 protoplasmic streaming in the phloem parenchyma remained unaffected while transport appears to have been checked. It may be noted that the concentrations of alcohol in the bark appear to be much lower than those that have a depressing effect on physiological processes (e.g. streaming, Seifriz (33)). It should be stressed that even if the check in transport was due to toxins, such an explanation would suggest the presence of a protoplasmic factor in transport rather than a stream of solution driven through a passive sieve-tube. It is noteworthy that, in spite of the isolation of bark and wood and the application of silica varnish to the outside of the bark, transport proceeded at approximately half the normal rate. One presumes that the exclusion of oxygen was not complete, or that it may be transported longitudinally through the phloem and/or the other tissues of the bark.

#### (B) *Experiment 6.*

##### (1) *Procedure.*

The plants were subdivided into regions as in the last experiment (see Fig. 17). There were three treatments and all had the Top region covered with metal cylinders. These cylinders were placed over the plants in the early stages of growth and were fixed into position with two corks when the experiment began. A thin layer of plaster of Paris was placed over the lower cork to prevent leakage of oil. All treatments were ringed at the base of the Bottom region. In the Normal group the bark was prised away from the wood as shown in the diagram. A strip of waxed paper was then inserted between bark and wood. The space between the stem and the cylinder was filled with moistened cotton wool. In the Oil group, mineral lubricating oil was poured into the cylinder to within about 2 cm. of the rim. This penetrated between bark and wood. Before use,

it was deoxygenated with moist iron filings under reduced pressure. In the Ringed group, additional rings of bark were removed as shown in Fig. 17. Half the plants in this group had their cylinders filled with oil and half were filled with moistened cotton wool. There were six plants per

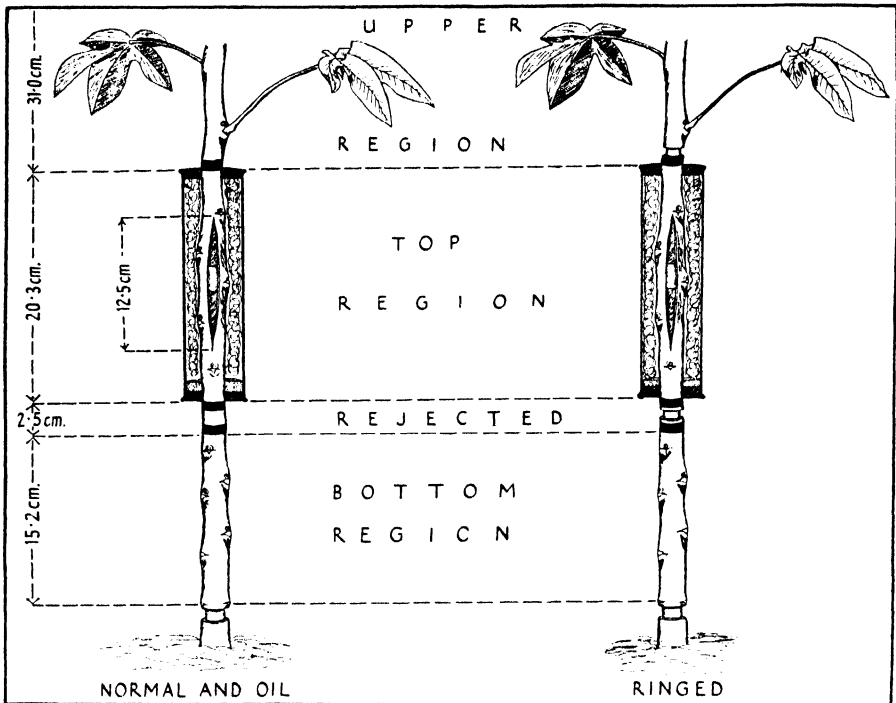


FIG. 17. Diagram Showing Treatment of Plants for Normal, Oil, and Ringed Groups (Experiment 6).

sample and three samples per collection. The results are expressed on the sample basis.

*Time-table.*

- |          |       |  |
|----------|-------|--|
| Dec. 10. | 1934. | Ringling of all plants below Bottom region (6 to 8 a.m.).                                  |
|          |       | Fixing of cylinders and making of additional two rings in Ringed group (8 a.m. to 2 p.m.). |
|          |       | Placing of oil or cotton wool in cylinders (2 to 4 p.m.).                                  |
| „        | 11.   | „ 1st collection of Normal, Ringed, and Oil groups.  |
| „        | 12.   | „ 2nd collection of Normal, Ringed, and Oil groups.  |
| „        | 13.   | „ 3rd collection of Normal, Ringed, and Oil groups.  |
| „        | 14.   | „ 4th collection of Normal, Ringed, and Oil groups.  |
| „        | 15.   | „ 5th collection of Normal, Ringed, and Oil groups.  |
| „        | 17.   | „ 6th collection of Normal, Ringed, and Oil groups.  |

(2) *Results.*

The dry weights of the Bottom region are shown in Fig. 18. The values for the individual samples are shown on the graph. At collections 1 and 2 the Oil group was down on the Ringed group, though the differences are not significant, while the Normal group showed fully significant increases on the Oil group. After the 2nd collection, recovery occurred in the Oil group and the pattern conforms rather closely with that of the Normal group. The Ringed group shows a negative but not significant correlation with time. *Protoplasmic streaming in the phloem parenchyma of the Top region of the Oil group proceeded unabated throughout the experiment.*

(3) *Discussion.*

This experiment supplies grounds for the belief that, provided the exclusion of oxygen is adequate, transport may be completely checked. This check occurs with great rapidity; the interval between the application of the oil and the first collection being only eighteen hours; no reserve of oxygen is consequently indicated. If we neglect the small differences at the first two collections between the Ringed and the Oil groups and calculate the extent of the check in the rate of transport, as we did in Experiment 3, we obtain the results shown in Fig. 19. It will be seen that there was a gradual recovery from the state of a complete block. Even at the time of the last collection the check is still greater than in any previous experiment. The fact of recovery, no doubt due to the oil gradually taking up oxygen, and the absence of any effect on streaming in the phloem parenchyma, make it clear that transport was not checked as a result of the presence of any toxic material in the oil. As streaming was not checked under the oil, even at collection 1, although it is stopped in an atmosphere of nitrogen, either the oxygen supply cannot have been completely curtailed or sufficient oxygen for streaming remained in the tissue. Longitudinal transport of oxygen through the phloem in amounts sufficient for transport would, however, seem unlikely.

## V. THE OVULE.

The experiments just described indicate that transport can be checked by exclusion of air from the bark, the extent of this check being dependent on the extent of the exclusion. Transport in these experiments was measured by the rate of accretion of dry matter in a region of stem separated from the foliage region by a region of bark from which air was partially or wholly excluded. They give, however, no indication of the extent to which the transport of the mineral elements was checked. In Experiment 2, it will be recollected, there was a suggestion that the transport of the mineral elements might be checked to a smaller extent than that of

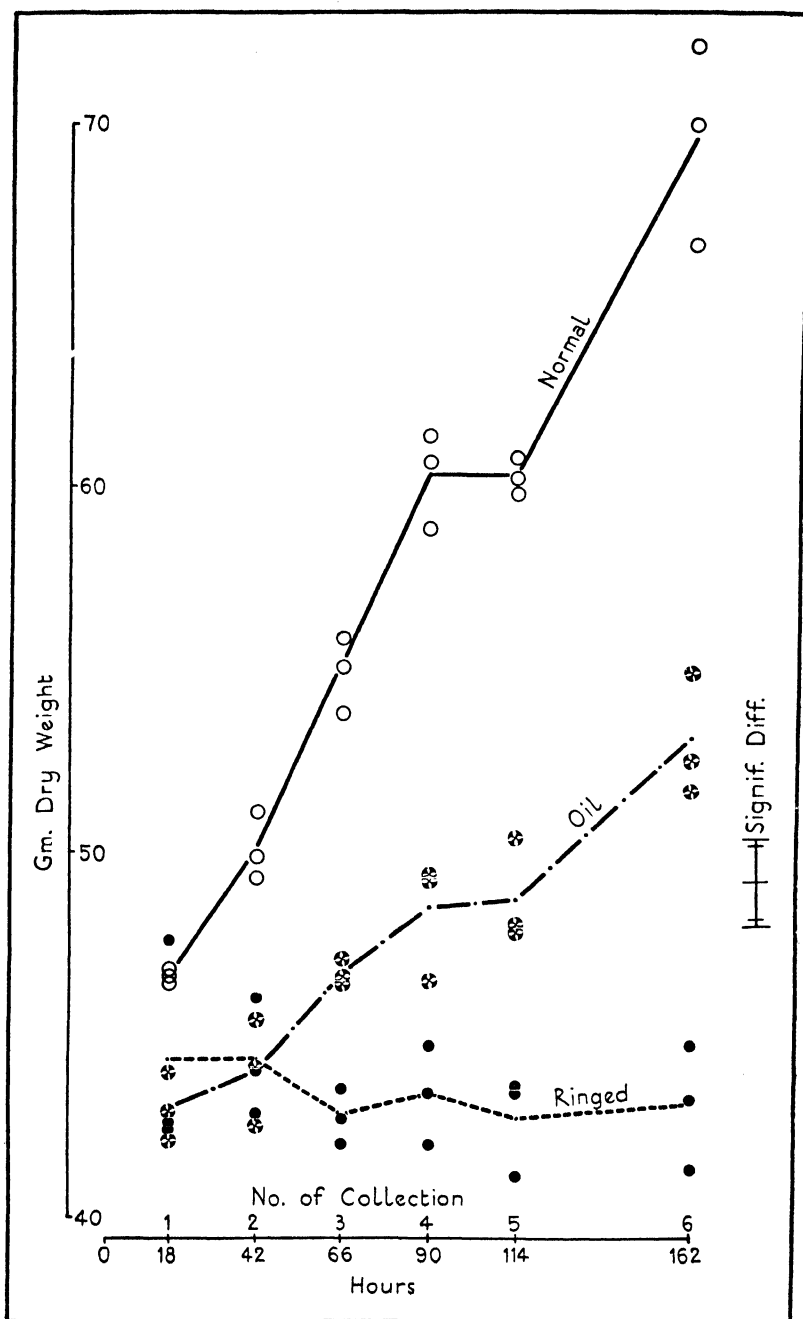


FIG. 18. Dry Weights of Bottom Region for Normal, Oil, and Ringed Groups (Experiment 6).

carbohydrate by this treatment. With a view to ascertaining whether or no this is so, we have utilized the boll as the sink and have measured the effect of excluding air from a region of defoliated fruiting branch on transport through it.

### *Experiment 7.*

#### *(1) Procedure.*

On December 22, 1933, about 2,000 flowers were tagged with wool. Between the flowers tagged and the main axis there were approximately 15 inches of fruiting branch (cf. Fig. 20). On January 3, 1934, all foliage leaves and bolls on the fruiting branches between the tagged bolls and the main axis were removed. The fruiting branches beyond the tagged bolls were cut away and the bracts removed from the bolls. Phloem mobile materials destined for the boll had thus to traverse a bared region of branch of about 15 inches. There were three treatments, and different treatments were situated on different plants. In addition to a Normal or Untreated group, there were two treatments in which a part of the fruiting branch was covered with plasticine. Plasticine was used here in preference to wax because it could be applied as a thick coat and because it remained unaffected by the sun. In the Short group, the coat of plasticine extended back from the boll for a distance of 6 inches, while in the Long group it extended backwards for a distance of 12 inches. There were two samples per collection and not less than fifty bolls per sample. As transpiration from the young ovule is very small (cf. 17, 21) and the bulk of the food materials arrive via the phloem, we consider only the changes that occurred in the ovule. The carpels were also sampled and their behaviour was found to accord very closely with that of the ovules. The results are expressed on the sample basis and represent the weights of materials found in the ovules of one hundred bolls.

### *Time-table.*

Dec. 22.	1933.	Flowers wooled.
Jan. 3.	1934.	Fruiting branches trimmed.
" 4.	"	Plasticine applied and initial collection made.
" 8.	"	1st collection of Normal, Short, and Long groups.
" 11.	"	2nd collection of Normal, Short, and Long groups.
" 15.	"	3rd collection of Normal, Short, and Long groups.

#### *(2) Results.*

The increments on the initial collection are shown in Table VI. The values recorded represent the weights of the various materials that arrived in the ovule during the course of the experiment. The increments for the Short and Long groups respectively are also expressed as percentages of

the increments for the Normal group. Taking first the results for carbohydrate,<sup>1</sup> and assuming that the same proportion of incoming carbohydrate was consumed in respiration in each of the three groups, it will be seen that in the Short group, at the 1st collection, the weight of carbohydrate

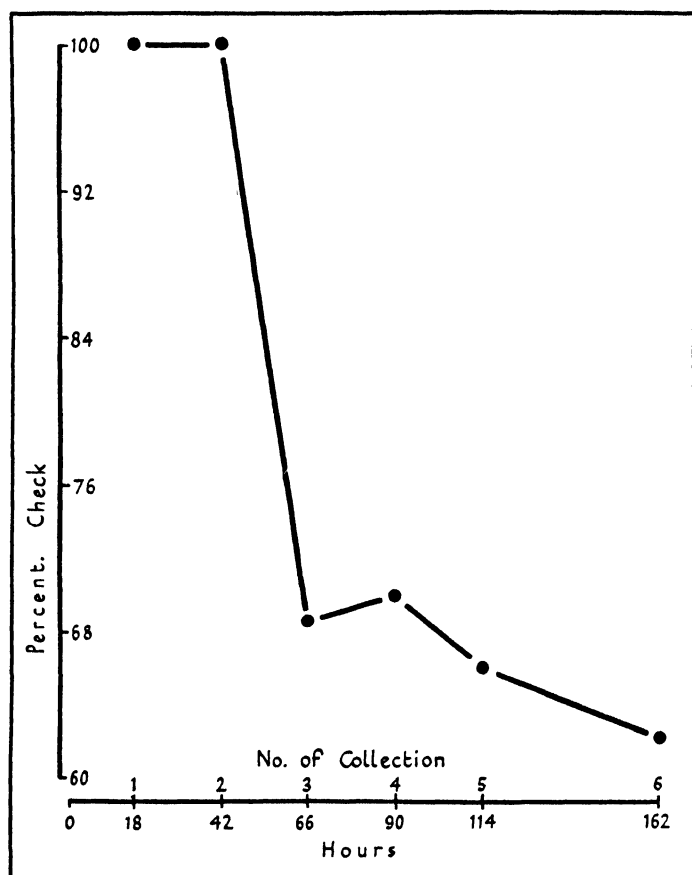


FIG. 19. Check in Transport for Oil Group, calculated from :

$$100 - \frac{\text{Oil} - \text{Ringed}}{\text{Normal} - \text{Ringed}} \cdot 100.$$

At collections 1 and 2 the negative values for Oil - Ringed are neglected (Experiment 6).

entering the ovule was only 57.1 per cent. of that arriving in the Normal group. The check in transport was, therefore, 42.9 per cent. At the 3rd collection, the weight of carbohydrate arriving in the ovule of the Short group was 59.9 per cent. of that arriving in the Normal group, and the check was 40.1 per cent. It would appear, therefore, that there was no increase over a period of eleven days in the extent to which carbohydrate

<sup>1</sup> Carbohydrate is calculated by deducting the weight of the mineral elements (5.7 N + 3.05 P + Cl + K + Mg + Ca) from the dry weight.

transport was checked. This is true also for nitrogen, phosphorus, potassium, and magnesium. Calcium may stand apart from the other elements in this respect, but, as it appears to be supplied to the ovule mainly via the wood (21), the question of a change in time in the extent to which its

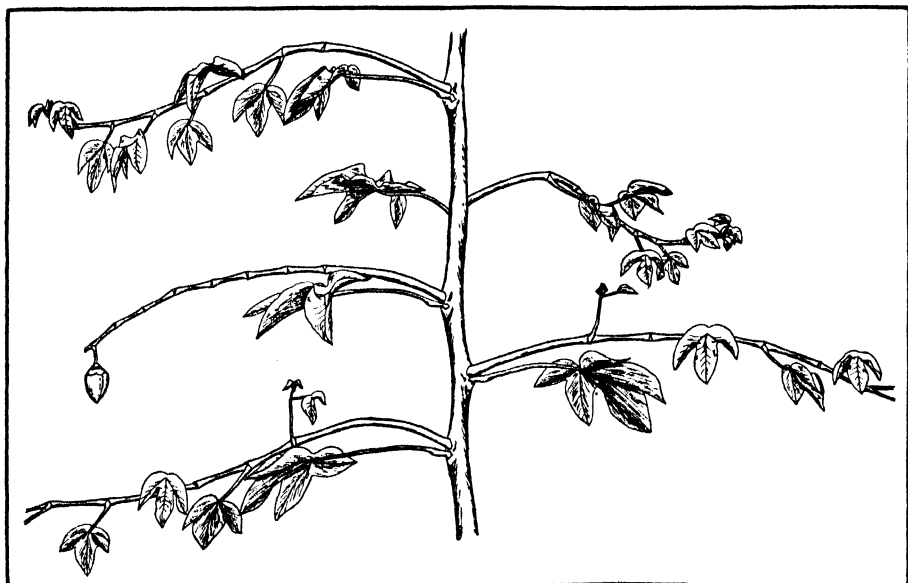


FIG. 20. Diagram showing Treatment of Fruiting Branch and Position of Boll (Experiment 7).

transport is checked does not arise. It may thus be concluded (cf. Experiment 6) that while the exclusion of oxygen causes a check in the rate of transport, the extent of the check need not increase in time. It will be seen, however, that in the Long group the extent of the check was greater at the 3rd than at the 1st collection. It is conceivable that this was due to the tendency of bolls to undergo abscission whenever growth is markedly checked. As we have already stressed, if the check in transport had been due to toxins liberated in anaerobic respiration, the extent of the check might have been expected to increase as the experiment progressed.

As to the behaviour of the various materials and the extent to which their transport was arrested by doubling the length of stem from which air was wholly or partially excluded, it will be convenient to consider the *mean* checks for all collections for each group. The check for each material is calculated by deducting the values given in columns 3 and 5 of Table VI from 100. The mean checks are shown in Table VII. It will be seen that the extent of the check was very similar for carbohydrate and nitrogen in both groups. Phosphorus, potassium, and magnesium also behave very similarly in both groups and differ from carbohydrate and nitrogen in that their transport was checked to a smaller extent than that of these materials.

TABLE VI.

*Increments (Gm. per Ovules in 100 Bolls) on Initial Collection for Normal, Short, and Long Groups with Increments for Short and Long Groups as Percentages of Increments for Normal Group.*

No. of collection.	Days after initial collection.	Material.	1.	2.	3.	4.	5.
			Normal.	Short.	Short as per cent. of Normal.	Long.	Long as per cent. of Normal.
1	4	Carbohydrate	22.80	13.03	57.1	8.67	38.0
		Nitrogen	0.618	0.341	55.2	0.243	39.3
		Phosphorus	0.113	0.071	62.8	0.053	46.9
		Potassium	0.477	0.350	73.4	0.282	59.1
		Magnesium	0.050	0.033	66.0	0.027	54.0
		Calcium	0.006	0.008	133.3	0.008	133.3
2	7	Carbohydrate	36.44	20.67	56.7	15.15	41.6
		Nitrogen	0.964	0.568	58.9	0.428	44.4
		Phosphorus	0.163	0.114	69.9	0.090	55.2
		Potassium	0.692	0.457	66.0	0.419	60.5
		Magnesium	0.083	0.058	69.9	0.045	54.2
		Calcium	0.032	0.030	93.8	0.023	71.9
3	11	Carbohydrate	61.75	36.97	59.9	19.69	31.9
		Nitrogen	1.501	0.857	57.1	0.468	31.2
		Phosphorus	0.273	0.187	68.5	0.117	42.9
		Potassium	1.076	0.737	68.5	0.435	40.4
		Magnesium	0.146	0.095	65.1	0.067	45.9
		Calcium	0.064	0.063	98.4	0.051	79.7

For calcium, there is no evidence that its transport was affected by the application of plasticine. For carbohydrate and nitrogen, transport was checked in the Short group by 42.1 and 42.9 per cent. respectively, and in the Long group by 62.8 and 61.7 per cent. respectively. The *mean* check for phosphorus, potassium, and magnesium for the Short group was 32.2 per cent. and for the Long group 49.0 per cent. Not only were these elements checked to a smaller extent than carbohydrate and nitrogen, but doubling the length of stem covered with plasticine has had a slightly smaller effect. This is due, no doubt, to the fact that while carbohydrate and nitrogen are supplied to the ovule almost exclusively via the phloem, an appreciable proportion of phosphorus, potassium, and magnesium is supplied through the wood (cf. 21). Similarly, the behaviour of calcium is due to the fact that it is supplied almost exclusively via the wood.

TABLE VII.

*Mean Checks for Short and Long Groups.*

Material.	Short.	Long.
Carbohydrate	42.1	62.8
Nitrogen	42.9	61.7
Phosphorus	32.9	51.7
Potassium	30.7	46.7
Magnesium	33.0	48.6
Calcium	-8.5	5.0



### (3) *Discussion.*

In this experiment, bark and wood in the region of stem covered with plasticine were in contact and both tissues were intact. It would thus appear possible that the check in transport, at least of the mineral elements, was due to a check in the rate at which the transpiration current travelled through the fruiting branch and not to any interference in the mechanism of transport in the phloem. Reference to Table VII shows, however, that the check was felt only by the phloem mobile elements, while calcium, which previous work has shown to be relatively immobile in the phloem, was delivered at approximately the same rate to 'plasticine' and normal ovules. This is in accord with previous work (21) and is of some interest in that it indicates that the movement of solution through the wood is in no way dependent on oxygen supply.

In Experiment 4 we found that banding a region of defoliated stem with rubber had a depressing effect on the rate at which dry material was accumulated by the plant. It was suggested that this might be due to the liberation of the products of anaerobic respiration into the transpiration stream, and that on their arrival in the lamina, their accumulation, possibly as a result of partial closure of the stomata, diminished the rate of carbon assimilation. It will be clear that respiration might have been activated rather than carbon assimilation retarded. As the uptake of minerals was found to be affected by excluding air from the petiole, this latter possibility is perhaps less likely than the former. In Experiment 6 we concluded that these same products of anaerobic respiration did not affect the mechanism of transport in the region where they actually originated.

In the experiment at present under consideration, it would appear possible, therefore, that the products of anaerobic respiration generated in the fruiting branch beneath the plasticine might have travelled to the ovule, possibly with the growth water, and that on arrival they might have interfered with the utilization of food materials, and thus have diminished the gradient of the mobile compound into the ovule. In the course of previous work (19) we concluded that sucrose was the form in which carbohydrate entered the ovule. Now, if the utilization of sucrose in the ovule had been affected as a result of the application of plasticine to the fruiting branch, the concentration of sucrose in the Long group should have exceeded that in the Short, and similarly the concentration in the Short group should have exceeded that in the Normal. The mean sucrose concentrations for the three collections in the ovule are shown in Table VIII. It will be seen that the concentrations are the exact opposite of what would have been expected if utilization of sucrose had been affected by the application of the plasticine. It may, therefore, be concluded that the carbohydrate metabolism of the ovule, like the transport mechanism in the phloem, remains unaffected by the products of anaerobic respiration. It would seem that the effect

in the leaf may be due primarily to some interference with stomatal regulation.

TABLE VIII.

*Mean Concentrations (Gm. per 100 Gm. Water) of Sucrose in Ovules.*

Group.	Sucrose.
Normal	0.74
Short	0.64
Long	0.50
Significant differences $\left\{ \begin{array}{l} P = 0.05 \\ P = 0.10 \end{array} \right.$	$\left\{ \begin{array}{l} 0.09 \\ 0.07 \end{array} \right.$

The difference suggested in Experiment 2 in the behaviour of the mineral elements and of carbohydrate, when the petiole is coated with wax, is not confirmed in the present experiment. The extents to which the transport of carbohydrate and nitrogen are checked are practically identical. The agreement is probably not accidental, though it must be recollected that the assumption is made that the proportion of carbohydrate entering the ovule which is respired is the same in the three groups. The further assumption is made that the proportion of carbohydrate used in supplying energy for transport is the same in the three groups. These assumptions are probably justified, and it may, we think, be concluded that the movement of carbohydrate and of nitrogen is checked to the same extent under conditions of oxygen starvation. The somewhat smaller checks of phosphorus, potassium, and magnesium may, as we suggested, be due to the fact that a greater proportion of these elements is supplied via the wood. If all these materials were checked to the same extent by a reduction in the supply of oxygen, it would indicate that they travel in the same channel, by the same mechanism, and that their movement is activated to the same extent by the energy released in respiration.

It remains to consider the effect of doubling the length of stem coated with plasticine on the amount of transport into the ovule. Nitrogen would appear to be the most satisfactory indicator of transport. Taking the mean nitrogen values for the three collections (Table VI) we found that for the Short group transport was checked (Table VII) by 42.9 per cent. and for the Long group 61.7 per cent. For every 100 gm. of nitrogen that arrived in the ovules of the Normal group 57.1 gm. entered the Short and 38.3 gm. the Long group. If movement had been accelerated in each of the two six-inch portions of the Long group to the same extent as in the Short group, the amount of nitrogen entering the ovules in the Long group would have been 57.1 per cent. of 57.1 or 32.6 gm. for every 100 gm. that entered the Normal group. Actually, 38.3 gm., which is in excess of the expected value of 32.6 gm., entered the ovules of the Long group. When, however, it is recollected that a check in transport must steepen the gradient across the

region covered with plasticine and that the greater the check the steeper will be the gradient, it is possible that the difference between the expected and actual values may be explained in terms of the alteration in gradient. It seems not unlikely, therefore, that the accelerating mechanism was slowed down to the same extent in both groups.

## VI. GENERAL DISCUSSION.

Our results confirm Curtis's conclusion that living cells are essential for transport. It would seem that solute movement through the sieve-tube is dependent on a continuous supply of oxygen. A part of the energy released in respiration must in some way expedite transport; a state of affairs that does not seem conformable with the existence of a *Druckstrom*. If, as appears to be the case, transport follows a diffusion pattern, and if, as also appears to be the case, streaming of the protoplasm does not occur in the sieve-tube, then the translocation of solutes must proceed through stationary cytoplasm and diffusion must be accelerated either by an increase in the free energy of the solute particles or by a diminution in the resistance of the solvent.

Theoretically, there would appear to be two general methods by means of which metabolic energy might be expended in activating diffusion. Firstly, it is possible to conceive of the solute particle itself utilizing the energy. Its free energy might be increased, resulting in what might be termed solute projection (cf. Dixon (8)), or its hydration might be in some way reduced. If the solute is projected, it might be imagined that rather high temperatures would prevail in the sieve-tube. For the present, however, it seems unnecessary to attempt to distinguish between the means by which the solute could utilize respiratory energy in accelerating its speed of movement. The second method by which energy might be spent in hastening diffusion involves the existence of some organization in the cytoplasm whereby the resistance offered to solute movement might be diminished. Something of this sort was suggested by Mason and Maskell (20) as a result of their work on carbohydrate transport. They state 'Thus, sugars appear to move in the sieve-tube under unit gradient at about the same rate as they would diffuse in air if they existed in the gaseous state. The question, therefore, arises whether any mechanism in the sieve-tube can be visualized which would reduce the resistance offered by the medium to diffusion. It is tempting to postulate the existence in the sieve-tube of some special organization of capillary structure which enormously reduces resistance to diffusion'. It would appear that the maintenance of such a special organization in the cytoplasm would require expenditure of metabolic energy.

To distinguish between these two ways in which energy might be expended, it is necessary to consider the consequences that might accrue

from its utilization by the solute and the medium respectively. If metabolic energy is utilized by the solute, it might be expected that only the movement of metabolites would be accelerated in the sieve-tube, and that the extent of the acceleration would depend on how intimately they enter into the metabolism of the cell. If, on the other hand, energy is utilized in the maintenance of some special organization in the cytoplasm, then the movement of all substances in solution in the sieve-tube should be accelerated and, moreover, be accelerated to the same extent.

We have found that sugars, nitrogen, phosphorus, potassium, magnesium,<sup>1</sup> and chlorine<sup>1</sup> are readily mobile in the phloem of the cotton plant, while calcium apparently is not. In an analysis of the exudate from the sieve-tubes of Cucurbit stems, we found the above materials to be present with the exception of calcium. It would appear that all materials in solution in the sieve-tube are mobile. The mobility of chlorine is of particular interest, for this element appears to be completely in solution and in an ionizable form in the cotton plant (24) and is usually considered to play no essential part in metabolism. Fluorescein, according to Schumacher (32), may travel through the sieve-tube at rates comparable with those of other solutes. Allusion may also be made to the work of Bennett (1), of Holmes (11), and of Caldwell (2) on the transmission of viruses through the phloem. It may, we think, be concluded that substances other than metabolites are freely mobile in the phloem. Moreover, the rate at which they move appears to be much greater than can be accounted for on purely physical grounds.

As to the extent to which the diffusion of various solutes is accelerated, we may refer to the work of Maskell and Mason (16). They say, 'the acceleration in the rate for unit gradient would seem to be about the same order for nitrogen as for sugars'. As sugar and nitrogen are both very intimately associated with metabolism, this result might perhaps be expected whether respiratory energy is utilized by the solute or by the medium. The position is perhaps different for nitrogen and magnesium. We found (cf. Table VII) that the transport of magnesium, in company with phosphorus and potassium, was checked to a smaller extent than that of nitrogen and carbohydrate, and suggested that this difference was due to the fact that a larger proportion of the phosphorus, potassium, and magnesium than of nitrogen and sugar entering the ovule was supplied by the transpiration current. An alternative explanation now presents itself, for this difference could arise if the acceleration of metabolites at any level of oxygen supply varied with their importance in the economy of the cell.

In Section V, it was found that when the length of stem coated with plasticine was doubled, the check in transport of nitrogen was increased from 42.9 per cent. to 61.7 per cent., so that for every 100 gm. of material

<sup>1</sup> Experimental data not yet published.

that traversed the stem of the Normal group, 57.1 gm. passed through the Short group and 38.3 gm. through the Long. It was pointed out that had the acceleration been the same in the two halves of the Long group as in the Short group, 57.1 per cent. of 57.1 or 32.6 gm. of material would have entered the ovules of the Long group. The value actually found, viz. 38.3 gm., is only a little greater than that expected, possibly, as we have suggested, as a result of the alteration in the gradient.

The two values are probably, therefore, sufficiently close to warrant the conclusion that the amount of material moved is reduced in geometrical progression when the length of stem coated with plasticine is increased. This would occur if the rate of oxygen supply by leakage through the plasticine was constant per unit of covered area, and if the accelerating mechanism worked independently of the amount of material moved. It is not what would obtain if there was a quantitative relation between oxygen supply and material in movement, for in this case the same amount of material should traverse both Short and Long groups. It does not indicate that metabolic energy is utilized by the solute, but rather that it is expended in maintaining some special state of the cytoplasm that permits solutes to travel at rates that are comparable with diffusion through a gas, and which is maintained whether transport is in progress or in abeyance.

## VII. SUMMARY.

1. When the petiole of a leaf was coated with paraffin wax, carbohydrate accumulated in the lamina in excess of the normal amount. This accumulation could be detected within twenty-four hours of the operation of applying the wax, but after a few days the amount declined below that in leaves whose petioles were not coated with wax. There was, at the same time, an accumulation of carbohydrate, phosphorus, and doubtless other phloem mobile mineral elements in the petiole. The interpretation of the experiment is complicated by the fact that there may be a great loss of all the phloem mobile mineral elements from the lamina. It is concluded that this type of experiment, in which the rates of carbohydrate production and of arrival of the mineral elements in the lamina are unknown, while leading to results that are in harmony with the view that curtailment of air supply to the petiole leads to a partial block in transport, is nevertheless ill-adapted to determining the role, if any, of oxygen in transport.

2. (a) A reduction in air supply to a region of bared stem may bring about a check (25 per cent.) in transport through it. On the other hand, transport may not suffer any check. It is considered possible that when oxygen supply through the lenticels is reduced, oxygen may be obtained from the transpiration current and that the extent of the check in transport depends on the oxygen-supplying power of the transpiration current.

(b) Growth in the region of stem from which air was excluded was checked to a much greater extent than transport through it. It is concluded from this that either the oxygen requirements for growth exceed those for transport or that the oxygen potential of the sieve-tube is stepped up as a result of the activity of the companion-cell. The compact nature of the phloem, and the localization of a peroxidase, leptomin, in the sieve-tube and companion-cell, prompts the suggestion that a carrier of oxygen may be a factor in the oxygenation of the sieve-tube.

3. Prising apart wood and bark over a short distance of stem and coating the inner and outer surfaces of the isolated bark with materials (e.g. vaseline, varnish, oil) to exclude air from it led to a great (up to 100 per cent.) reduction in the rate of transport through it.

4. (a) Observations were made on the effect of a thick coat of plasticine applied to a completely defoliated branch, on the rate of transport of carbohydrate and a number of mineral elements into the young seeds situated in a fruit at the end of the branch. Two lengths of branch were covered with plasticine, a Short group of 6 inches and a Long one of 12 inches. It was assumed that the movement of water through the xylem would be greatly reduced as a result of defoliation.

(b) It was found that the movements of carbohydrate and of nitrogen were equally checked, and that the movement of phosphorus, of potassium, and of magnesium were also checked to about the same extent as one another, but that the check for the first group was somewhat greater than that for the second. Possible explanations of the difference are discussed.

(c) It was also found, taking nitrogen as the indicator of transport, that doubling the length of stem covered with plasticine diminished the amount of material transported in approximately geometrical progression. It is pointed out that this would occur if the degree of oxygen starvation was the same throughout the length of stem covered with plasticine, and if the acceleration of diffusion was the same in the two halves of the Long group as in the Short one. It is suggested that the mechanism activating diffusion consists in some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve-tube at rates comparable with those in a gas.

#### ADDENDUM.

As pointed out in an earlier paper (22) no calculation of the energy needed to expedite diffusion in the sieve-tube seems possible. At first sight it would appear that the energy requirements would be prodigious and could not be furnished by the carbohydrate present. It should, however, be stressed that the relation of solute to solvent in the cytoplasm and

in water may be different. There are some grounds for the belief that the solubility of sucrose in the sap varies from one tissue to another. Moreover, little as yet seems to be known concerning the combination of simple diffusion processes with surface conditions (cf. M. H. Jacobs, *Ergeb. der Biologie*, xii, 1-160, 1935, and M. Volmer, *Trans. Far. Soc.*, xxviii, 359-63, 1932) and nothing is known concerning the combination of simple diffusion processes with surfaces kept active by respiration (cf. H. F. Clements, *North West Science*, viii, 9-21, 1934).

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# A Fossil Dicotyledonous Wood from Assam.

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With Plate VII.

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## I. INTRODUCTION.

IN a recent note (4) it was reported that the Forest Research Institute Fossil No. 2 had been collected by Mr. C. S. Purkayastha of the Assam Forest Service, and sent to the Forest Research Institute, Dehra Dun, for identification. During his tour in the Nowgong Forest Division, he came across this fossil near Nailalung Railway Station, 14 miles east of Lumding Junction on the Assam-Bengal Railway. The specimen is 5 inches in diameter and about 8 inches in length. Its preservation has been very bad and uneven. The black stripes seen on the longitudinal surfaces indicate slight deterioration of the wood prior to fossilization. The fossil consists mainly of coarse, slightly ferruginous, yellow to sometimes brown sandstones. According to Evans (8), the locality from which the specimen was collected, is of the Tipam Sandstone Stage. He says that in this Stage fossil woods are not 'very abundant except in small area east and south-east of Lumding'. In the opinion of the Geological Survey of India this fossil is 'probably of the Middle Tertiary Age'. Further geological detail of this area will probably be available when the Geological Survey

[*Annals of Botany*, Vol. L. No. CXCIX, July, 1936.]

of India, which is at present carrying out a survey in Assam, completes the work.

In a previous note (4), as stated above, affinities of the Forest Research Institute Fossil No. 2 to the living *Glutas* were reported, and the name *Glutoxylon assamica* was proposed for it. In the present paper it is the intention to compare anatomically this fossil with the living dicotyledons as well as with the dicotyledonous fossil woods that have been so far recorded.

## II. DESCRIPTION OF THE F.R.I. FOSSIL NO. 2 AND ITS COMPARISON WITH THE LIVING DICOTYLEDONS.

### A. *Macroscopic Study.*

*Description of the fossil:* a diffuse porous wood.

*Growth rings:* The metatracheal parenchyma bands may give the impression of growth rings, but their distribution is too irregular to be taken for the marks of true growth rings.

*Vessels:* distinctly visible to the eye, medium-sized to large, appearing as white dots against the ground-mass of the wood, single or in radial pairs, oval, tylosed, occasionally filled with brownish deposit; vessel lines distinct on the longitudinal surfaces due to whitish contents of the vessels.

*Soft tissues:* in the well-preserved portion of the specimen they are found in a row round the vessels, but not distinct in the badly preserved portion. They are also found in thin metatracheal bands which are very irregularly distributed. Some of the bands are very near each other—as many as three bands in a millimetre are not uncommon; others may be wide apart—as much as 3 mm. in between two bands. These bands are either continuous or end abruptly after running a short distance (Pl. VII, Fig. 1).

*Rays:* fine, scarcely visible to the eye but distinct with a lens, more or less evenly distributed; on the radial surface they are visible but not prominent. Ripple marks have not been observed.

*Gum ducts:* disintegration of woody tissues may give the impression of vertical gum ducts in a row, but it is doubtful whether this is the case. Horizontal gum ducts are not visible with the eye or hand lens.

The wood collections, both Indian and foreign, of the Forest Research Institute, Dehra Dun, have been examined with a view to finding out timbers which depict macroscopic anatomical structure similar to that of the F.R.I. Fossil No. 2. In this connexion, various publications on plant anatomy, especially Den Berger (6), Foxworthy (10), Gamble (11), Garratt (12), Lecomte (16), Moll and Janssonius (17), Pearson and Brown (18), have also been referred to. The list given below shows the families and genera of the woods which show some affinities (in gross anatomy) with the fossil in question.

1. Guttiferae.

*Calophyllum* Linn.

2. Anacardiaceae.

*Bouea* Meissner; *Gluta* Linn.; *Melanorrhoea* Wall; *Mangifera* Linn.; and *Swintonia* Griff.

3. Leguminosae.

*Hardwickia* Roxb. (specially *H. pinnata* Roxb.; and *H. alternifolia* Elmer.); *Kingiodendron* Harms.; and *Sindora* Miq.

4. Myristicaceae.

*Myristica* Linn. (specially *M. amygdalina* Kurz.; and *M. irya* Gaertn.); and *Horsfieldia* Willd.

B. Microscopic Study.

*Description of the fossil.*

*Growth rings*: indistinct (Pl. VII, Fig. 1).

*Vessels*: solitary or in radial pairs of 2-9 (mostly 2-4), less frequently in tangential pairs, profusely tylosed, occasionally filled with solid deposit (Pl. VII, Figs. 1, 3); perforation-plate horizontal or slightly oblique, simple; vessel elements tailed or not (Pl. VII, Fig. 5). Inter-vessel pits large, alternate, border oval, when crowded hexagonal, widest horizontally; aperture lenticular, horizontal or slightly oblique (Pl. VII, Figs. 5, 7, 9). Vessel-ray pits very large, oval or round, one to a few per ray cell, with wide aperture and narrow border, horizontally aligned (Pl. VII, Fig. 5). Vessel-parenchyma pits are of the same type as those of vessel-ray but vertically aligned.

*Fibres*: difficult to distinguish individually in badly preserved portion of the wood, but where the preservation is fairly good they are thin to thick walled, somewhat tangentially compressed, in radial rows. Interfibre pits moderately numerous, small, mostly confined to radial walls, border small; aperture slit-like, vertical or slightly oblique (Pl. VII, Fig. 2).

*Parenchyma*: scanty; (1) paratracheal, in a sheath of 1-2 cells, seldom forming eyelets (Pl. VII, Figs. 1, 3); (2) metatracheal band, thin, 1-4 cells wide (mostly 2), (Pl. VII, Fig. 3); continuous or ending blindly, spaced irregularly (Pl. VII, Fig. 1); (3) diffuse not observed, probably due to bad preservation. Interparenchyma pits numerous, fairly large, oval, rarely grouped.

*Rays*: two types; (1) simple, 1-2 seriate (mostly 1) (Pl. VII, Fig. 4), heterogeneous, intercellular spaces not distinguishable. Individual cells squarish to oblong in tangential section (Pl. VII, Fig. 4). (2) Fusiform with horizontal gum ducts, 2-4 cells wide, gum duct mostly one in a ray,

seldom two (Pl. VII, Figs. 4, 6); distribution irregular, but always present; epithelial cells present, small, in a row round the duct. Ray cells in radial section mostly procumbent, rarely square or upright.

*Gum ducts*: vertical type not observed. From the above description, it will be noticed that the fossil shows one anatomical feature of diagnostic importance, i.e. horizontal gum ducts. According to Den Berger (6), Garratt (12), Lecomte (16), Moll and Janssonius (17), Pearson and Brown (18), Record (19), Solereder (24), and also as a result of microscopic anatomical study by the author, out of eleven genera which have been found to show some similarity in gross anatomy with the fossil, only three genera of the Anacardiaceae have horizontal gum ducts. In view of this, it does not appear to be worth while to study microscopically all the eleven genera. Only the anatomical structures of *Gluta*, *Melanorrhoea*, and *Swintonia* of the Anacardiaceae have been given here, and their similarity and dissimilarity with that of the fossil have been discussed.

TABLE I.

*Comparison of Anatomical Structures of the Fossil with those of Gluta, Melanorrhoea, and Swintonia.*

	Vessels. <sup>1</sup>	Fibres. <sup>1</sup>	Parenchyma. <sup>2</sup>	Rays.
F.R.I Fossil No. 2.	Moderately thick walled.	Thin to thick walled.	(1) Paratracheal sheath of 1-2 cells. (2) Metatracheal bands of 1-4 cells (mostly 2). (3) Diffuse not observed.	(1) Simple, 1-2 se- riate (mostly 1). (2) Fusiform, 2-4 se- riate with hori- zontal duct.
<i>Gluta</i> .	Moderately thick walled.	Thin to thick walled.	(1) Paratracheal sheath of 1-3 cells. (2) Metatracheal bands of 1-4 cells (mostly 2-3). (3) Diffuse scanty.	(1) Simple, 1-2 se- riate (mostly 1). (2) Fusiform, 2-4 se- riate with hori- zontal duct.
<i>Melanorrhoea</i> .	Moderately thick walled.	Thin to thick walled.	(1) Paratracheal sheath of 1-3 cells. (2) Metatracheal bands of 1-10 cells (most- ly 3-7). (3) Diffuse very scanty.	(1) Simple, 1-2 se- riate (mostly 1). (2) Fusiform, 2-4 se- riate with hori- zontal duct.
<i>Swintonia</i> .	Thin walled.	Very thin walled.	(1) Paratracheal sheath of 1-3 cells and ex- tending in wing- like fashion. (2) Metatracheal bands of 2-8 cells (mostly 3-5). (3) Diffuse scanty.	(1) Simple, 1-3 se- riate (mostly 2). (2) Fusiform, 3-4 se- riate with hori- zontal gum duct.

<sup>1</sup> See Chattaway (3).

<sup>2</sup> See Record (19).

It may be noted here that these anatomical characters have been found to be constant in the different wood specimens of the same species, as well as in the different species of the same genus except in *Melanorrhoea*.

The fossil differs from the *Swintonias* in the distribution of vertical parenchyma cells, in the width of the ray cells, and in the thickness of walls of fibres and vessels. In view of these dissimilarities, it is most unlikely that the fossil is one of the *Swintonias*. Out of the remaining two genera, *Gluta* and *Melanorrhoea*, the latter exhibits certain anatomical variation within the genus. This variation has been found to be confined to the distribution of metatracheal bands, based on which the genus may be divided into two main groups. Those that have thick bands of 1-10 cells (mostly 4-7) and those that have thin bands 1-6 cells (mostly 2-4). Of these two groups the latter is very much like *Gluta*, and from an anatomical point of view it appears to be convenient to link up this small group of *Melanorrhoeas* to the *Gluta*. Finally, from the above anatomical study, it would appear that the fossil in question is one of the *Glutas* (including those *Melanorrhoeas* which have thin metatracheal parenchyma bands).

### III. COMPARISON OF THE F.R.I. FOSSIL NO. 2, WITH THE DICOTYLEDONOUS FOSSIL WOODS PREVIOUSLY IDENTIFIED.

#### A. Fossils from India.

At the outset it may be mentioned that so far only a few dicotyledonous fossil woods from India have been identified (see Sahní (21) and Seward (23)). The first one was done by Holden (14) in 1916. She found a fossil from the Irrawady (Tertiary) Series of Burma possessing anatomical characters similar to those exhibited by the members of the Dipterocarpaceae. The name *Dipterocarpoxyton burmense* was therefore given by her. Later on, Bancroft (1) doubted the validity of Holden's identification, and pointed out the probable affinities of this fossil to the Meliaceae. Recently Gupta (13), who had re-examined Holden's original specimen, named it as *Irrawadioxyton burmense*. According to him it does not exhibit any affinities to the Dipterocarpaceae, but 'shows closer resemblance with the wood of the Ebenaceae and the Anacardiaceae'. In this connexion Gupta discusses very briefly some ebenaceous woods, namely, *Diospyros embryopteris*, *D. macrophylla*, and *D. virginianum*; and some anacardiaceous woods, *Gluta tavoyana* and *Glutoxyton assamica* Chowdhury (a note on the diagnostic anatomical structure of the F.R.I. Fossil No. 2 was recently published by the author of this paper, and the name *Glutoxyton assamica* was proposed in it), and he reports their affinities to this Irrawadian fossil. It is not at all clear how Gupta found the anatomical characters of Holden's *D. burmense*, similar to those of the recent *Diospyros* and *Gluta*, which differ so much from each other. In Table II it will be seen that the

anatomical differences between *Diospyros* and *Gluta* are so distinct that there is little likelihood of confusing them for the purpose of identification.

TABLE II.

Genera.	Vessels.	Rays.	Parenchyma.	Pits.
<i>Diospyros</i> .	Small to medium sized.	One type : (a) 1-2 seriate (mostly 1).	(a) Paratracheal. (b) Regular meta-tracheal band of 1-2 (mostly 1) forming reticulum with the rays.	(a) Inter-vessel small, oval to hexagonal, with lenticular aperture. (b) Vessel-ray numerous per ray, oval, with lenticular aperture and wide border. (c) Vessel-parenchyma, same as (b).
<i>Gluta</i> .	Medium sized to large.	Two types : (a) simple, 1-2 seriate (mostly 1). (b) Fusiform with gum duct, 2-4 seriate.	(a) Paratracheal. (b) Irregular meta-tracheal band of 1-4 (mostly 2-3).	(a) Inter-vessel large, oval to hexagonal, with lenticular aperture. (b) Vessel-ray a few per ray, very large, oval, round to rectangular, with thin border and large aperture. (c) Vessel-parenchyma, same as (b).

The anatomical data of Holden's *D. burmense* given by Gupta (13) are incomplete, and cannot be profitably compared with Table II. Gupta did not record the pits on the various types of cells of the fossil. He mentions that they are simple, but this is not enough for the identification of a timber. Besides the question of pitting, the other anatomical structure of the fossil seems to be similar to that of the *Gluta*. It will, however, be necessary once again to re-examine Holden's original specimen with a view to finding out the pitting on different types of cells. If the pits are found to be similar to those of the *Gluta* it will come under *Glutoxylon*. On the other hand, if the fossil shows similarity to *Diospyros* in pitting as well as in other respects, an appropriate name will have to be given to it. In any case, there does not appear to be any justification to keep it under the present artificial genus *Irrawadioxylon*, which was applied by Gupta to indicate affinities to both *Diospyros* and *Gluta*. In view of the marked difference in the anatomical feature of *Diospyros* and *Gluta*, the fossil will have to be re-examined for its systematic classification and adequate naming.

Another fossil wood from the Lalmai hills of Bengal was reported by Sen (22) as *Dipterocarpoxyylon* type. He found this fossil 'resembling in every respect to Holden's *Dipterocarpoxyylon burmense*', but he did not report its complete anatomical data. Considering the present systematic position of Holden's original specimen, it would appear to be worth while to re-examine Sen's specimen for its proper identification.

## B. Fossils from Outside India.

Amongst the fossil woods identified from outside India, *Dipterocarpoxyton annamense* Colani (2) is of interest. Colani reported resemblance of her fossil with Holden's *Dipterocarpoxyton burmense*, and named it as a Dipterocarpoxyton. But it may be pointed out here that the photomicrographs and the description of the fossil given in her paper show its general affinities to the F.R.I. Fossil No. 2. In view of the present systematic position of Holden's specimen and of the resemblance of this fossil from Indo-China to the F.R.I. Fossil No. 2, it would appear advisable to re-examine it in order to place it in its legitimate systematic position.

An anacardiaceous wood from Caucasus was reported by Felix (9) and named as *Anacardioxyton uniradiatum*. But this wood is different from F.R.I. Fossil No. 2 in not having metatracheal parenchyma bands and horizontal gum ducts. Considering these anatomical differences, they do not appear to be in any way directly related.

Another anacardiaceous wood from South Sumatra was reported by Krausel (15) as *Anacardioxyton molli*. Later, Den Berger (5) re-examined it, and gave the name of *Sumatroxyton molli*. On careful examination of this fossil, Den Berger was not sure whether it belonged to Anacardiaceae or Burseraceae, as some members of both these families have horizontal gum ducts. To be on the safe side he gave the name *Sumatroxyton molli*. However, this fossil from Sumatra has simple rays of 2-3 seriate and no metatracheal parenchyma. These anatomical differences are enough to show that it is entirely different from the F.R.I. Fossil No. 2. Thus it will be seen that the anacardiaceous fossil woods so far reported do not show any great affinity to the fossil in question.

## IV. NAME OF THE FOSSIL AND ITS DIAGNOSIS.

In the earlier part of this paper it has been shown that the F.R.I. Fossil No. 2 shows all diagnostic anatomical structures similar to those of the Gluta and a few Melanorrhoea species. The generic name Glutoxyton, which was proposed in a previous paper by the author (4), includes all Gluta and those Melanorrhoea which have thin metatracheal bands of parenchyma, but excludes the Melanorrhoea with thick metatracheal bands. It will be noticed that no attempt has been made to relate the fossil to any living species of the Gluta or the Melanorrhoea, for at the present stage of our knowledge of the anatomical variability within the species such an action will not serve any useful purpose. All that has been done is that the fossil has been referred to a form-genus, and that the specific name *Glutoxyton assamica* is confined to the individual specimen, but not in the sense used by the systematic botanists. The generic and specific diagnoses of the fossil are given on next page.



*Genus* : *Glutoxylon*, K. A. Chowdhury<sup>1</sup> in Curr. Sci. 3 (1934), pp. 255-6.

A diffuse porous wood.

*Growth rings* : indistinct.

*Vessels* : medium sized to fairly large, visible to the eye, solitary or in radial pairs of 2-9 (mostly 2-4), less frequently in tangential pairs; profusely tylosed, occasionally filled with solid contents; perforation plate simple, horizontal or oblique; vessel elements with or without tail; inter-vessel pits large, alternate, widest horizontally, border oval, or hexagonal when crowded; aperture lenticular, horizontal or slightly oblique; vessel-ray pits very large, round to oval, one to several per ray cell, with wide aperture and narrow border, horizontally aligned; vessel-parenchyma pits are of the same type as those of vessel-ray but vertically aligned.

*Fibres* : thin to thick-walled, somewhat tangentially flattened, in radial rows, occasionally filled with solid contents. Pits mostly on the radial walls, small, round border, and slit-like aperture.

*Parenchyma* : scanty; just visible to the eye, distinct with a lens; (1) paratracheal, in 1-2 rows of sheath, often interrupted by rays; (2) metatracheal thin band of 1-4 cells (mostly 2), some continuous, others end blindly, irregularly spaced; (3) diffuse not observed. Inter-parenchyma pits numerous, fairly large, oval, rarely grouped.

*Rays* : fine, scarcely visible to the eye, distinct with a lens, moderately numerous, heterogeneous, occasionally containing solid deposits; (1) mostly uniseriate, rarely biseriate, individual cells squarish to oblong in tangential section, intercellular spaces not discernible; (2) fusiform with horizontal gum duct, 2-4 seriate, gum duct mostly 1 in a ray, rarely 2, distribution irregular but always present; epithelial cells present in a row round the duct.

*Gum ducts* : vertical type, not observed.

*Species* : *Glutoxylon assamicum*<sup>2</sup> K. A. Chowdhury; *Glutoxylon assamica* K. A. Chowdhury; in Curr. Sci. (1934), pp. 255-6.

*Vessel* : scanty, somewhat evenly distributed, usually 55-6 (82), single or groups per 50 sq. mm.; of these about 30 per cent. in pairs; moderate-sized to rather large; tangential diameter of solitary vessels  $183 \pm 30 \mu$ , radial diameter  $247 \pm 34 \mu$ ; vessel elements short to very long.

<sup>1</sup> This diagnostic description is according to the suggestions made by Chattaway (3).

<sup>2</sup> The specific description of the fossil is in accordance with the standard method of describing wood proposed by Chattaway (3), Rendle and Clarke (20). Due to bad preservation the minimum number of counts necessary for each anatomical structure of the fossil was not possible to obtain. Figures included in the above description are based on counts as shown below :

Vessel distribution, 20 counts; tangential and radial diameter of vessel, 101; vessel length, only 5; fibre length, 50; uniseriate ray width and height, 50; fusiform ray width and height, 25.

*Fibres* : short,  $975 \pm 62 \mu$  in length.

*Rays* : numerous  $9 \pm 1$  per mm. ; (1) uniseriate type extremely fine,  $30 \pm 15 \mu$  in width, in height more than 15 cells, and  $299 \pm 43 \mu$  ; (2) fusiform type with gum ducts fine to moderately broad,  $65 \pm 16 \mu$  in width ; in height more than 15 cells, and  $321 \pm 36 \mu$ .

#### V. SUMMARY.

1. A dicotyledonous fossil wood from Nailalung, Assam, is described ; its anatomical structures with those of the living dicotyledons are compared, and its affinities mainly to the *Gluta* are shown.

2. Detailed description of the genus *Glutoxylon* and of the species *G. assamicum* are recorded.

3. Some of the fossil woods so far reported from India and outside India are discussed ; their anatomical similarity and dissimilarity with *Glutoxylon assamicum* are noted.

Acknowledgements are due to Mr. S. S. Ghosh, of the Forest Research Institute, Dehra Dun, for general help in the laboratory ; to Mr. C. E. Parkinson, Forest Botanist, Forest Research Institute, for many helpful discussions ; to Professor B. Sahni, of Lucknow University, for allowing the author the facilities of his library ; and to the Director, Geological Survey of India, for information regarding geology of the fossil.

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### EXPLANATION OF PLATE VII.

Illustrating Mr. Chowdhury's paper on 'A Fossil Dicotyledonous Wood from Assam'.

(All photomicrographs are from untouched negatives.)

Fig. 1. Transverse section showing vessels and unequally spaced metatracheal parenchyma bands.  $\times 25$ . (This is a negative print.)

Fig. 2. Portion of a fibre showing characteristic pits on the radial wall.  $\times 190$ .

Fig. 3. Transverse section showing a thin metatracheal band composed of two rows of parenchyma cells (s) and tylosed vessel with adjacent ray (r).  $\times 210$ .

Fig. 4. Tangential section showing uniseriate rays and a fusiform ray (fr) with gum duct.  $\times 50$ .

Fig. 5. A vessel element showing vessel-ray pits (vrp) and inter-vessel pits (ivp).  $\times 190$ .

Fig. 6. Tangential section showing a fusiform ray with gum duct.  $\times 210$ .

Fig. 7. Intervessel pits highly magnified.  $\times 650$ .

Fig. 8. Portion of a ray showing pits on the end walls of the cells.  $\times 400$ .

Fig. 9. Portion of a vessel showing intervessel pits. Note hexagonal shape of the pits when crowded.  $\times 190$ .





# The Anatomy of a Cone-bearing Axis of *Lepidodendron Wortheni* Lesquereux.

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With seven Figures in the Text.

*LEPIDODENDRON WORTHENI* Lesquereux has been known from impression material since 1866. It was first described by Lesquereux (6) from North America and was later reported from France by Zeiller (10) and from various coalfields in this country by Arber (1, 2, 3, 4). In England it occurs in the upper and middle coal measures, and has been found in the coalfields of Cumberland (1), the Forest of Dean (Gloucestershire) and Somerset (2), the Wyre Forest (Worcestershire) (3), and Staffordshire (4). The literature, however, contains no record of its internal structure.

The following account of the anatomy of this fossil is based on a series of sections (seventeen transverse and ten longitudinal) cut from a cone-bearing axis found in the Yorkshire coalfields at Barnsley and identified by the late Dr. Kidston as *L. Wortheni* Lesquereux. The cone itself was unfortunately decayed, but the preservation of the stem was quite good.

I am indebted to Professor H. S. Holden for the opportunity of examining these slides and for his helpful criticism and advice.

In transverse section the stem has a diameter of 16 mm. and is nearly circular. Tissue differentiation is similar to that of other *Lepidodendra*. The following structures are present:—

1. A polyarch stele.
2. A cortex divisible into an inner and an outer zone.
3. A phelloderm.
4. An outer covering of leaf bases.
5. Numerous leaf traces (see Fig. 1)

These parts are described and briefly discussed.

1. *The stele.* The stele is placed somewhat excentrically. It is small, approximately 2 mm. in diameter, and shows no secondary growth. It consists of a central medulla surrounded by a zone of well-preserved xylem, which is separated by a space from the surrounding phloem. Numerous leaf traces arise from the xylem (Fig. 1).

The greater part of the medulla is composed of thin-walled parenchymatous cells, but small groups of thick-walled cells of tracheid form occur

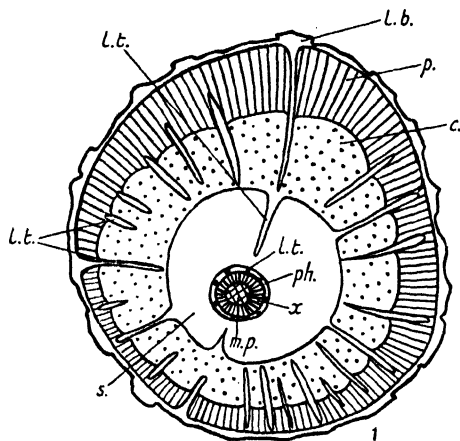


FIG. 1.

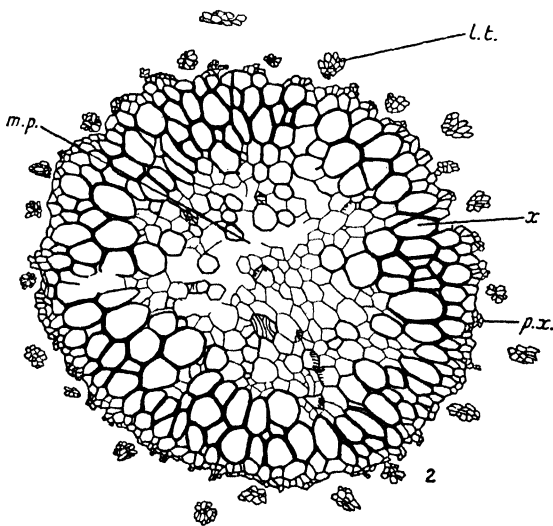


FIG. 2.

FIGS. 1 and 2. Fig. 1. Diagrammatic transverse section of stem of *Lepidodendron Wortheni*. Fig. 2. Transverse section of xylem showing leaf traces and mixed pith. *c* = cortex, *l.b.* = leaf base, *g.* = ligule in pit, *l.t.* = leaf trace, *m.* = parenchymatous cells, *m.p.* = mixed pith, *p.* = phloem, *ph.* = phloem, *p.x.* = protoxylem, *s.c.* = secretory cells, *s.* = space in cortex, *s.s.* = space in stele, *t.* = tracheids, *x.* = xylem. (This lettering is used in these and the succeeding figures.)

evenly distributed among them (Fig. 2). In longitudinal section (Fig. 3) these tracheids are seen to be arranged in vertical series. They are short, of about the same length as the parenchymatous cells, and have their walls

thickened with scalariform bands. Reticulate thickenings are characteristic of the horizontal cross walls of these cells. The thickenings of the cell-walls are connected at right angles by numerous much finer bands (Fig. 3), and it has been suggested that similar fine connecting bands in other

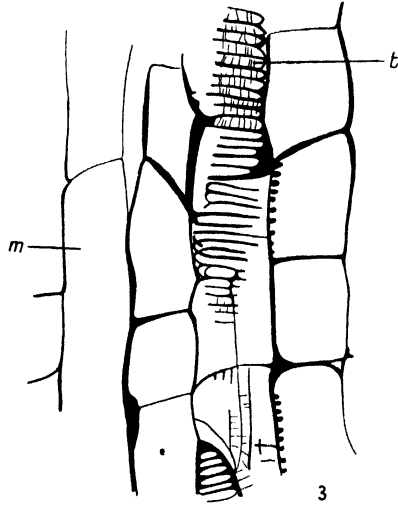


FIG. 3.



FIG. 4.

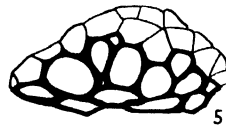


FIG. 5.

FIGS. 3-5. Fig. 3. Longitudinal section of medulla showing the two types of cell. Fig. 4. Transverse section leaf trace immediately after leaving the xylem. Fig. 5. Transverse section leaf trace in phloem showing associated thin-walled tissue.

Lepidodendra may have been produced by the shrinkage of the unthickened parts of the cell-wall on drying, or that they may represent a stage in the absorption of the wall (Seward (9)).

The peripheral primary xylem is composed entirely of tracheids. Each tracheid has an irregular polygonal cross-section and the majority are large and radially elongated, but on the outer margin of the cylinder there are a few rows of smaller elements. The protoxylem groups are about twenty-five in number and they project as small ridges from the main body of xylem, giving it, in transverse section, a crenulate margin (Fig. 2). A noticeable feature of the sections is the presence of narrow spaces between many of the xylem elements which give the effect of pale lines running between the dark cell-walls. Possibly these spaces were



formed by the disintegration of the middle lamellas before or during petrification. Longitudinal sections show that the tracheids are thickened in a scalariform manner, but, in contrast to the tracheids of the medulla, they are long cells with oblique cross walls. The protoxylem elements are narrower than those of the metaxylem and have spiral or annular thickenings.

No cell-structure remains of the ground tissue which immediately surrounded the xylem, but in this region there are numerous leaf traces which appear to have left the xylem at the protoxylem groups. They consist of xylem only (Fig. 4).

The phloem, which has been moderately preserved, is made up of a ground tissue of thin-walled cells among which dark secretory cells occur. The latter form an irregular ring in the outer part of the tissue, and they also encircle the outgoing leaf traces. The inner layers of phloem are composed of radially flattened cells, but towards the outer margin, in the region of the secretory cells, the elements are more nearly isodiametric. Longitudinal sections show that the cells of the phloem are elongated, two or three times as long as broad, and that they have horizontal or only slightly oblique cross walls. The dark secretory cells occur in vertical series.

2. *The cortex.* Apart from the leaf traces no cellular structure has been preserved in a broad zone to the outside of the stele. Presumably the tissue which originally occupied this space was delicate and formed the inner and middle cortex which characterize other *Lepidodendra*. The outer cortex of the specimen is, in parts, quite well preserved. It is a compact tissue consisting mainly of rather thick-walled, isodiametric, empty cells, which decrease in size towards the outer edge. At intervals the outer cortex is cut across radially by leaf traces and the cortical cells which sheath the trace, by the spaces once occupied by parichnos strands, and, where the section has just missed a leaf trace, by narrow bands of radially elongated parenchymatous cells. On the inner margin of the cortex are two or three rows of radially-flattened thin-walled cells, which in longitudinal section show a marked difference from the elements constituting the main part of the tissue. They are very short and have horizontal cross walls, while the thick-walled cells are elongated and have obtusely-pointed ends, which dovetail together. In the better preserved parts of the specimen a narrow band of dark secretory cells occurs almost immediately outside the radially-flattened cells, and in many cases they also flank the outgoing leaf traces. Where preservation is less good the cortical cells are found separated from one another embedded in a structureless black matrix.

3. *The phelloderm.* The phelloderm, which forms a band of tissue outside the primary cortex and immediately inside the leaf bases, is in this

specimen, unequally developed on opposite sides of the stem. At frequent intervals it is broken by radial gaps, often continuous with the spaces in the outer primary cortex, which appear in some cases to be due to outgoing leaf traces and in others to poor preservation. The phelloderm cells are arranged in regular radial rows and are rectangular in transverse section, while in radial and tangential sections they appear as long narrow elements with rather blunt ends. The outermost few layers of cells have thicker walls than the rest of the tissue and are frequently separated by a space or by a band of crushed cells from the inner thinner-walled portion. This break in the continuity of the phelloderm possibly marks the original position of the phellogen, and if this is so the meristem was far more active on its inner than on its outer side. There are, however, some portions of the phelloderm which show no such break and in which the thin-walled type of cell changes abruptly to the thick-walled type. In the outer part of the phelloderm some indications of secretory cells occur.

4. *The leaf bases.* The leaf bases are poorly preserved, but in some cases a compact undifferentiated tissue of rather thick-walled parenchymatous cells remains. The cells are approximately isodiametric and polygonal in transverse section, two or three times as long as wide, and have obtuse ends. Some epidermal cells of approximately cubical form have been preserved. One ligule, consisting of rather thick-walled parenchymatous cells is quite well preserved and, as seen in transverse section, is triangular in outline and lies in a small triangular cavity (Fig. 7).

5. *The leaf traces.* From an examination of the radial longitudinal section it appears that the endarch leaf traces leave the xylem cylinder at the protoxylem groups. When detached they consist entirely of scalariform and spiral tracheids and are approximately circular in transverse section (Fig. 4). They make their way almost vertically upwards into the phloem, at the same time becoming radially flattened. In the phloem a strand of thin-walled cells attaches itself to each trace on the abaxial side (Fig. 5) and though this delicate tissue is poorly preserved, there are

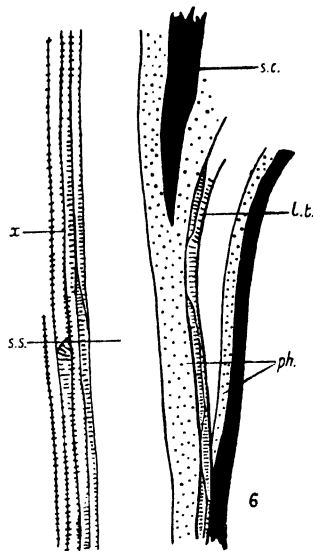


FIG. 6.

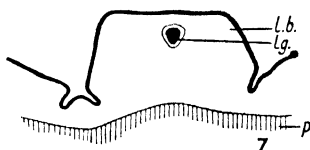


FIG. 7.

FIGS. 6 and 7. Fig. 6. Diagrammatic longitudinal section of leaf trace leaving the stele showing formation of phloem foliar gap. Fig. 7. Diagrammatic transverse section leaf base showing ligule in ligular pit.

indications that it consisted of fairly long cells with nearly horizontal cross-walls. The collateral trace was probably surrounded by a small amount of thin-walled tissue, for usually it lies in a small cavity. At the point where a leaf trace leaves the stele the phloem bulges slightly into the adjoining space and the trace is seen in transverse section to be surrounded by a ring of dark secretory cells. This is due to the fact that a phloem foliar gap is formed by each trace (Fig. 6).

After passing the phloem a trace follows an almost horizontal course through the space originally occupied by the inner and middle cortex, and though the xylem is the only well-preserved part, remains of thin-walled cells accompanying the wood can be distinguished. On reaching the outer cortex it makes a sharp upward bend through an angle of approximately  $60^{\circ}$ . A wide space on the lower side of the trace shows that a parichnos originally accompanied it into the outer cortex, where there are again indications of a thin-walled tissue composed of rather long narrow cells on the abaxial side of the xylem. In the phelloderm the traces return to more nearly horizontal courses, and finally they pass out into the leaf bases at right-angles to the surface of the stem, but preservation in this region is so poor that no details are distinguishable, and the course of the parichnos cannot be followed.

#### DISCUSSION.

From the above account it is evident that the structure of *L. Wortheni* resembles that of *L. selaginoides*. The presence of short tracheids in the medulla of *L. Wortheni* is an interesting feature, since up to the present time *L. selaginoides* was the only species known to possess a central column of mixed tracheids and parenchyma. The remaining preserved structures of the stele of *L. Wortheni* appear to agree in all particulars with the corresponding structures of *L. selaginoides*.

According to Scott (8), twigs of *L. selaginoides* 1 cm. in diameter may show secondary thickening, but this specimen of *L. Wortheni*, although 1.6 cm. in diameter, shows no secondary growth in the stele.

No comparison can be made between the inner and middle cortex of the two species, but the outer primary cortex and the phelloderm are similar. A characteristic feature of old stems of *L. selaginoides*, i. e. those showing secondary growth in the stele, is the presence of concentric rows of secretory cells in the phelloderm. Mention has been made of the presence of secretory cells in the outer part of the phelloderm of *L. Wortheni*, and though they are not numerous, this is probably because the stem is a young one.

The leaf traces in both species are endarch, in this differing from *L. Harcourtii*, which has mesarch traces, and their behaviour as they pass outwards is similar.

From a single specimen it is impossible to draw any definite conclusions as to the exact relationships of this fossil, but as the parts preserved so closely resemble the corresponding parts of *L. selaginoides* it is certain that *L. Wortheni* is nearly related to *L. selaginoides*.

#### SUMMARY.

The anatomy of *L. Wortheni* Lesquereux, hitherto known only from impression material, is described and its affinities discussed.

Representative sections of this fossil have been given to the Geological Survey and to the Natural History Museum.

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## Notes on Conifers.

### X. Some Normal and Abnormal Structures in *Taxus baccata*.

BY

W. T. SAXTON.

With five Figures in the Text.

A RECENT volume by Chamberlain (1) has prompted the publication of the remarks and observations following. Chamberlain draws a comparison between *Taxus canadensis*, investigated rather fully by Dupler (3), and *T. baccata* which was studied by several of the older morphologists, and especially by Hofmeister (4), Jaeger (5), and Strasburger (12). He states that whereas in *T. canadensis* all the contents of the pollen tube enter the archegonium, in *T. baccata* only the functional sperm does so. Fig. 1, of *T. baccata*, shows a fertilized archegonium where both the stalk and tube nuclei are very clearly seen within it. Though the small non-functional sperm was not identified it seems scarcely likely that it alone is left behind, and we may surely conclude that there is no essential difference between the two species (or sub-species?) in this respect.

Chamberlain also remarks that the prothallus in *T. canadensis* is already cellular throughout when the pollen tube reaches it. No doubt this is sometimes the case, but Dupler (3) states (p. 119) that the pollen tube frequently reaches the female gametophyte when the latter still consists of free nuclei. This is likewise the case in *T. baccata*, where the pollen tube, more often than not, is in contact with the young prothallus some time before cell formation begins when only 32 or 64 nuclei are present, or exceptionally even less. Dupler figures two examples of this kind in *T. canadensis* (3, Figs. 28 and 30).

Chamberlain further states that 'in *Taxus baccata* several investigators have failed to find more than one megaspore germinating'. He does not mention who the 'several investigators' are, but he has evidently overlooked the figure of *T. baccata* given by Mirbel and Spach (6) which shows this phenomenon, as well as the records of Hofmeister (4), Jaeger (5), Strasburger (12), and Miss Robertson (9), all of whom record this fairly common occurrence. The writer has seen the same thing in various preparations of *T. baccata*. It is of interest to notice that the allied genus *Austrotaxus* (11) shows the same peculiarity. Indeed, Chamberlain's list of examples might have been considerably extended.

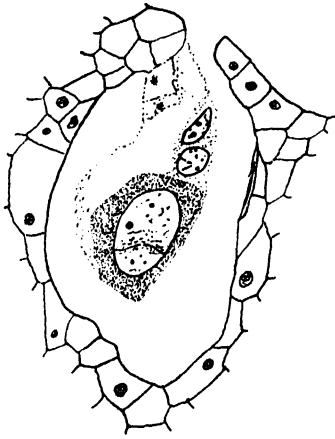


FIG. 1.

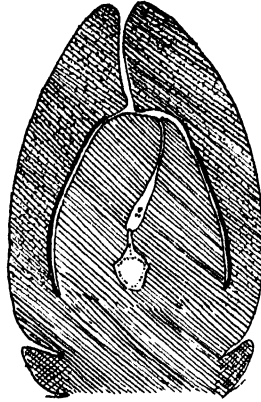


FIG. 2.

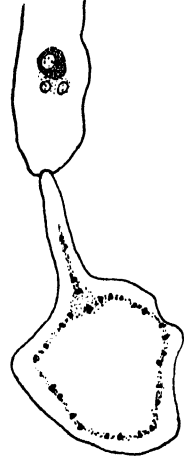


FIG. 3.

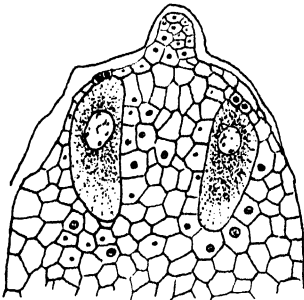


FIG. 4.

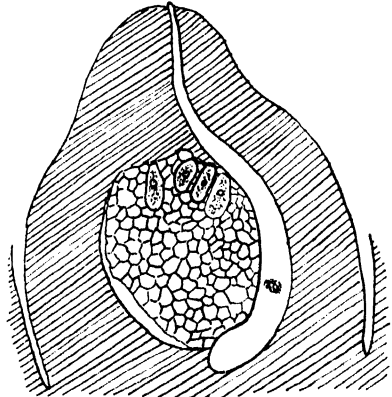


FIG. 5.

FIG. 1. Longitudinal section of a fertilized archegonium of *Taxus baccata*, showing the male and female nuclei in contact, and the stalk and tube nuclei above.  $\times 400$ .

FIG. 2. Longitudinal section of a young ovule of *T. baccata* showing position of pollen tube, young prothallus, and 'prothallial tube'.  $\times 25$ .

FIG. 3. Central part of the preceding; all the prothallial nuclei are in pairs with the remains of the spindles of the last division between them.  $\times 125$ .

FIG. 4. Longitudinal section of the upper end of a prothallus with fully formed archegonia, and showing a well-developed 'tent-pole' structure at the apex.  $\times 125$ .

FIG. 5. Longitudinal section of an abnormal ovule; for description see text.  $\times 40$ .

Figs. 2 and 3 show an exceptionally clear example of a rather common phenomenon; the young prothallus has itself put out an upward growing tube, which has met the advancing pollen tube in the nucellar tissue. This case is only exceptional in the length of the 'prothallial tube'; a rather shorter one is often seen. Although Dupler says almost nothing about this feature in *T. canadensis* it is evident from his figures 28, 47, 50, and 53 that it is about equally prevalent in the American species. Traces of a similar structure are shown by Coulter and Land in *Torreya taxifolia* (2, Fig. 15). There can be little doubt that this, at least in some cases, is the way in which the apical 'tent-pole' shown in Fig. 4 may arise. Something of this kind was observed by Sahni (10) in *Cephalotaxus*, but the figure he gives makes comparison difficult. A similar but less pronounced example was figured by the writer (11) in *Austrotaxus*. It would seem, therefore, to be an occasional occurrence in all Taxaceae. The interest of the 'prothallial tube' lies in the fact of a possible comparison with the unique 'prothallial tubes' of *Welwitschia* (7).

Fig. 5 shows a rather remarkable abnormality. A pollen tube has grown down alongside the prothallus, ending immediately below it, with the body cell and sterile nuclei about three-quarters of the way down and a long way from any of the archegonia. The latter have extended somewhat laterally, but the displacement is not great. No other example of this kind has been seen, but a very similar figure is given by Dupler in *T. canadensis* (3, Fig. 29). It is somewhat striking that even an occasional abnormality of this kind should be so closely duplicated in the two forms.

The preparations illustrated here were made some seven or eight years ago, partly in an attempt to discover any trace of a ventral canal nucleus. None was found, and as far as the writer is aware none has ever been seen in any form of *Taxus*. The only other case where it is believed that no such nucleus is formed is *Torreya taxifolia* (2), but in this case it has been definitely identified in an allied species of the same genus (8), so that *Taxus* is the only genus in which there is good evidence that it is never formed.

It may be remarked that the points taken up here have a wider significance than appears at first sight. In the large majority of conifer genera a good deal is now known of the life-history in at least one species, but there are very few genera where sufficient is known of two or more species to make it clear whether the details discovered in one species apply also to the rest of the genus. But in those few genera the details of structure and development of the gametophytes and pro-embryo have proved, with rare exceptions, to be of generic, and not merely specific, import. Those rare exceptions, again, concern mainly plants not invariably recognized as co-generic, such as *Wellingtonia* (*Sequoia*) *gigantea* and *Sequoia sempervirens*, *Thuja occidentalis* and *Biota* (*Thuja*) *orientalis*. Facts such



as these tend to emphasize the importance of gametophyte characters and details of embryogeny in questions of phylogeny and classification; it is reassuring to find that the various alleged differences set forth by Chamberlain between two species (or sub-species) so closely related as *T. baccata* and *T. canadensis* do not, in fact, exist.

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# Tissue Differentiation in Some Foliage Leaves.

BY

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With Plates VIII and IX and fourteen Figures in the Text.

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## INTRODUCTION.

AN accurate knowledge of the internal structure of foliage leaves was made possible by the introduction of practical improvements in the microscope in the early part of the nineteenth century. Before this date most of the records are concerned with the surface tissues of the leaf, and these are of great interest from an historical point of view. Hooke, in 1665, in his 'Micrographia', published drawings of the epidermal cells and of the hairs on the under surface of the stinging nettle, and Malpighi, ten years later, was the first person to see and to figure the stomata of a leaf. After this there was a gap of more than one hundred years before the next important contribution appeared. This was the publication in 1827 of de Candolle's 'Organographie Végétale' (1). In this work de Candolle described the upper and lower epidermis and an intermediate zone between them to which he first gave the name 'mesophyll'. He thought the mesophyll was made up of two systems, one of which received the ascending sap and brought it into contact with the air, and a second system which received the elaborated food and conducted it to the stem where it was used for nutrition. De Candolle did not distinguish between these two systems anatomically. Later, Trécul (11) and Eichler (3), both published work on

[Annals of Botany, Vol. L. No. CXCIX. July, 1936.]

the development of foliage leaves, but they were concerned with the sequence followed in the formation of the various parts of the mature foliage leaf, i.e. blade, petiole, &c., and the internal structure during development was not touched upon by them.

The publication of 'The Origin of Species' in 1859 provided a great incentive to botanists to examine plant tissues in detail and so to test the evidence provided by macroscopic observations of the adaptation of plants to their environments. The result of this stimulus was the publication of a considerable amount of work on the anatomy of various parts of the mature plant. The anatomy of mature foliage leaves was the subject of researches by Schwendener (9) and Haberlandt (4, 5). Schwendener (9) concentrated on the mechanical principles exhibited by the foliage leaves of monocotyledons, while Haberlandt in 1884 classified leaf tissues on a physiological basis. The latter worked out the arrangement of the tissues of mature leaves to illustrate the adaptation of leaves to their physiological needs. In the course of this very detailed work, Haberlandt described the anatomy of the leaves of many genera showing various forms of mature structure. His work demonstrated that, from a purely anatomical point of view, the mature foliage leaf was a very efficient organ for performing the function of photosynthesis. His interpretation of the varieties in leaf structure was based on two principles which were held to govern the construction of the photosynthetic system, (1) the principle of the exposure of the maximum amount of wall surface to the action of the light, and (2) the efficient translocation of the products of carbohydrate metabolism away from the leaf. Also, the degree of development of the photosynthetic system was thought to be regulated by the light intensity. He says, 'This power of accommodation (i.e. to differences in light intensity) depends on the fact that light has a direct influence upon the photosynthetic cells, in the sense that an increase in the intensity of illumination not only permits of increased photosynthetic activity, but also results in a more extensive development of photosynthetic tissue'. In a short section of an earlier paper he made a few observations on some of the earlier stages of development of the leaves of *Ficus elastica*, *Sambucus nigra*, and *Caragana frutescens*. The most important point mentioned was that, in the palisade layer of these examples, cell division occurred mostly by means of walls laid down in a radial direction.

More recently, Schuepp (7, 8) has published work on the development of the leaf of *Acer pseudoplatanus*, but his interest centres mostly in the contribution of the various meristematic layers of the apex towards the formation of the young leaf; he makes few observations about the differentiation of the leaf tissues themselves. He makes it clear, however, that one method of division predominates in the cells of the mesophyll; they divide almost entirely in the direction parallel to the surface of the leaf.

The stages in the development of the different tissues of the leaves of *Victoria plum* have been worked out by the author in connexion with an investigation into the cytology of the leaves of this tree when attacked by *Stereum purpureum*—the cause of 'Silver Leaf' disease of *Victoria plum* trees. A knowledge of the stages of development in normal leaves was needed to interpret the reaction of the leaves to the disease. While this piece of research was in progress it was found that very little work had been published on the stages of differentiation of leaf tissues; the work was, therefore, continued on the same lines, but using a wide range of material. A knowledge of the cytology of foliage leaves, it was hoped, would lead to a fuller understanding of the subject as a whole, also it might be of value in interpreting the sequence of events in the attacks of fungal diseases to which many foliage leaves are liable.

There is a large variety in the mature structure of the foliage leaves of Angiosperms and Gymnosperms, but they are all developed from meristematic tissue separated off from the growing point. These leaf primordia are potentially equivalent as far as we know, but the story of their differentiation in the various species indicates a wide range of possibilities inherent in them. When, in the developmental history of the various types of foliage leaves, do the differences of structure become apparent, and what differences in development underlie the differences found in the mature structure? Also, can the differences in shape of the cells forming the same tissue in the leaves of different species be correlated in any way (1) with the time of development and the relative amount of development of the various tissues, (2) with the differences in rate of nuclear division in the various leaf tissues, (3) with the type of metabolism of the cells of the different tissues, (4) with external conditions? These are some of the problems of leaf development which seemed to be outstanding, and the work was planned with these problems as a background against which to interpret the developmental story of the species selected.

In this preliminary paper the development of the leaves of the following eight species will be described to illustrate some of the methods of development to be found in foliage leaves: (1) *Victoria plum*, (2) *Sambucus nigra*, (3) *Pinus sylvestris*, (4) *Alstroemeria aurantiaca*, (5) *Ginkgo biloba*, (6) *Erica baccans*, (7) *Rosmarinus officinalis*, (8) *Salix alba*. It is hoped that later this will be supplemented by accounts of the development of the leaves of other species of plants so as to cover more fully the range of leaf development to be found in Angiosperms and Gymnosperms.

The tissues which are to be found in mature foliage leaves can be divided into the following classes:

1. Mesophyll tissues, comprising the cells responsible for the main photosynthetic activities of the leaf.
2. Epidermal tissues, e.g. epidermal cells, cuticle, stomata.

3. Vascular tissue.

4. Hypodermal tissue.

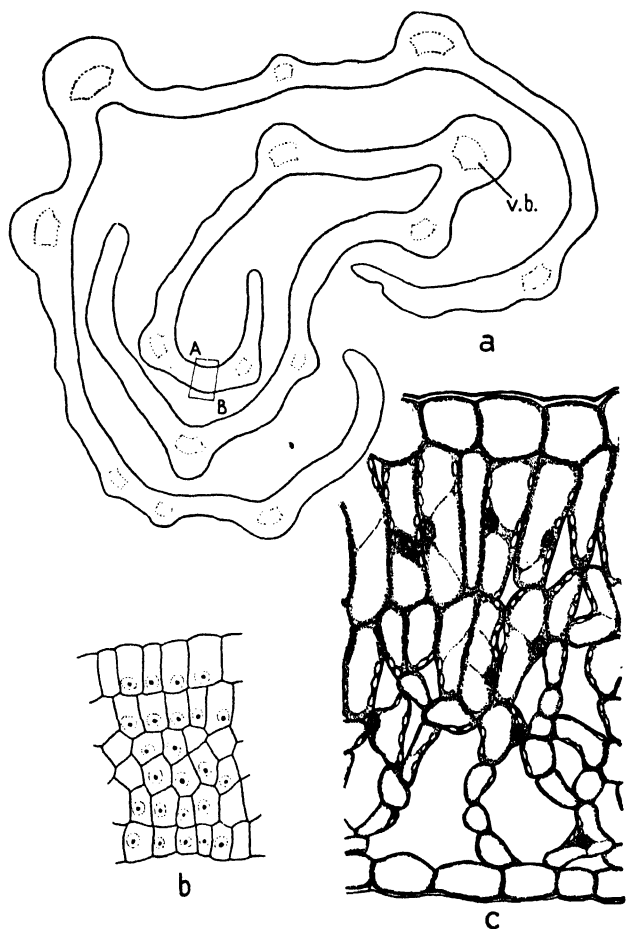
The cells forming these tissues all pass through various stages of differentiation before they reach maturity. In the earliest meristematic stage they are thin walled and full of dense protoplasm in which is embedded a comparatively large nucleus; it is at this stage that cell division is of frequent occurrence. Priestley (6) has stressed the fact that the metabolism of this type of cell is mainly protein in character. The cells next begin to increase in size, vacuoles filled with cell sap gradually appear in the protoplast; the cell walls stretch and the protoplasm, which does not appear to increase in amount, is pressed against the wall owing to the osmotic pressure of the cell sap in the vacuoles. The cells at this stage are still capable of division, but it is not of such frequent occurrence as it is in meristematic cells. The metabolism of the cell now tends towards the production of carbohydrates, and it is possible that this change in the metabolism may initiate the stretching stage of cell differentiation. After this stage—which varies very much in different tissues—has been passed through, the cell wall tends to become more rigid, and in many tissues it is thickened by the deposition of cellulose, suberin, cutin, &c., before it finally reaches maturity.

The foliage leaves of Gymnosperms and Angiosperms are organs of limited growth and, until chloroplasts are developed and are functioning, they are dependent on external sources (i.e. the vascular supply of the stem) for the whole of their food supply. The differentiation of the cells comprising the different tissues, therefore, involves a competition for the available food supply between the developing tissues in the early stages of their growth. The cells of these tissues may pass through the succeeding stages of development at very different rates or at approximately the same rate, but, in either case, the development of the leaf as a whole involves a complicated inter-relationship between the developing tissues, and the resulting mature structure of the leaf is the expression of the balanced nature of this relationship.

#### VICTORIA PLUM.

The plum leaf may be considered to be a typical dorsiventral leaf, and the stages of its development have been described by the author in a previous paper (10). The most important features of its development may be summarized as follows: While folded within the bud, and in the earliest stages after the bud has opened, the mesophyll consists entirely of meristematic cells showing no differentiation between the cells of the future palisade and spongy mesophyll. The upper epidermal cells vacuolate at an early stage, while those of the lower epidermis remain meristematic for a considerably longer period, during which time the stomata are formed.

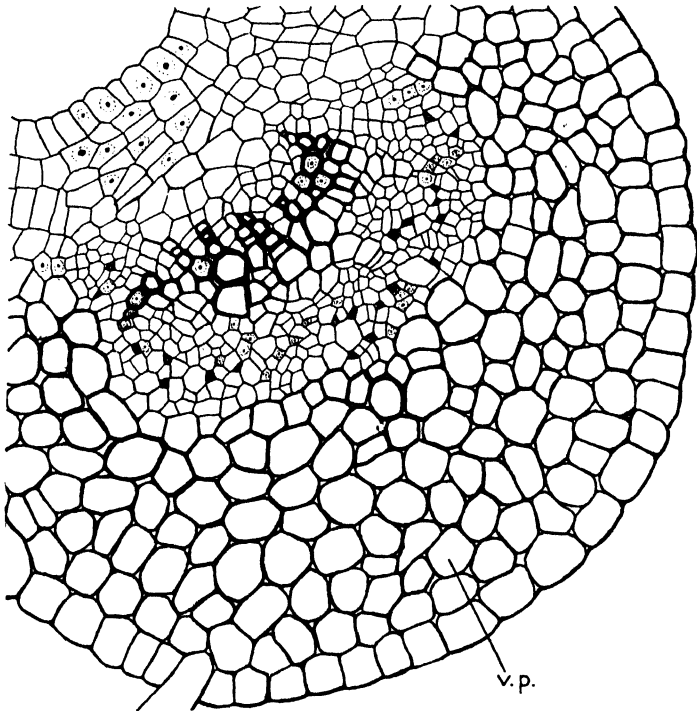
The vascular supply is the first tissue to become differentiated as a procambial strand, and its development proceeds rapidly and gives rise to a network of veins in the meshes of which the mesophyll is developed. To make the summary as complete as possible, diagrams showing some of the



TEXT-FIG. 1. *Victoria Plum*. *a*. Transverse section of leaves in bud,  $\times 32$ . *b*. Part of leaf AB,  $\times 310$ . *c*. Part of mature leaf,  $\times 310$ . v.b. = vascular bundle.

most important characteristics have been made, but more detailed drawings are to be found in the author's previous paper to which reference should be made. Text-fig. 1, *a* shows a transverse section of two young leaves at the stage when the bud is beginning to unfold. The vascular supply of these leaves is well developed, and vascular parenchyma has been formed in considerable quantity below the main bundles (Text-fig. 2). The mesophyll, however, still consists of meristematic cells, but the upper epidermal cells have already vacuolated. No stomata and no intercellular space

system is present at this stage (Text-fig. 1, b). The next stage is that of a leaf soon after it has left the bud. In such a leaf the difference in shape between the cells of the palisade and the spongy mesophyll is more noticeable, nuclear divisions are still taking place in the palisade cells, but



TEXT-FIG. 2. *Victoria Plum*. Transverse section of main vascular bundle of young leaf,  $\times 310$ . v.p. = vascular parenchyma.

they are less frequent in the spongy mesophyll cells. The palisade cells are characterized by a high rate of nuclear division in which all the new walls are laid down at right angles to the surface of the leaf. The characteristic palisade-shaped cells are present before the leaf opens out in the light, suggesting that the rate of nuclear division in these cells is a more important factor in producing the palisade tissue than the contact of the layer with light.

When the leaf is fully differentiated, the stretching stage in cell differentiation has been passed through, producing the very elongated palisade cells and the rounded, irregularly shaped cells of the spongy mesophyll, between which the intercellular space system has been developed in connexion with the stomata (Text-fig. 1 c). The mesophyll cells differentiate in the meshes of the vascular supply, the cells of which form resistant lines of tissue from the apex of the leaf to the base. The upper epidermal

cells too complete their differentiation before those of the mesophyll. This relatively early differentiation of the vascular supply and the upper epidermis compels the walls of the cells of the palisade to stretch mostly in the direction at right angles to the surface of the leaf. Since the cells of the spongy mesophyll are not so numerous and not closely packed, they are free to expand into almost any shaped cells, and they form bridges of cells across the enlarged space below the palisade cells. The intercellular space system is dependent for its development on the extent of the stretching stage of the cells of the spongy mesophyll. The first intercellular space is that formed below the young stomata, and it is during the development of the stomata that the stretching stage is initiated and the intercellular spaces are formed.

Some very marked change in cell dynamics evidently takes place when the cell walls begin to stretch. This phase may be initiated by the development of chloroplasts, which can be distinguished when the cells begin to vacuolate, and when functioning they would presumably produce a big rise in the rate of carbohydrate metabolism in the mesophyll cells of the young leaf, and so form a supply of energy to enable such comparatively rapid change in cell form to take place.

Although the stages of cellular differentiation are passed through at different rates in the various tissues of such a typical dorsiventral leaf, the development of these leaf tissues appears to take place simultaneously throughout the leaf and no localized meristematic region is present.

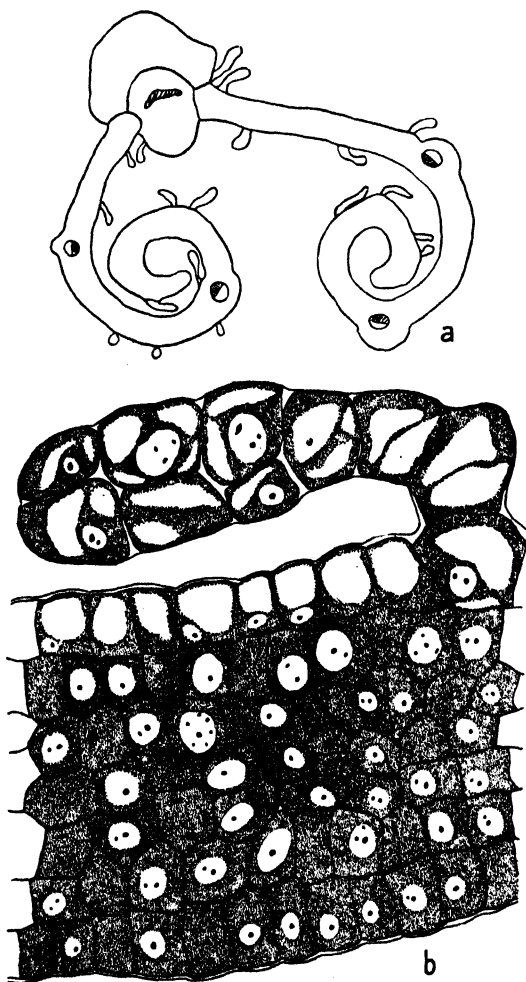
#### *SAMBUCUS NIGRA.*

In external appearance the leaf of *Sambucus nigra* would be regarded as a typical dorsiventral compound leaf, but its development shows many interesting points of contrast with that of the leaf of *Victoria plum* just described.

In the earliest stages, just after the leaf has emerged from the bud, the leaflet is narrow, elongated, and has a rolled-in edge; it is very soft to touch and of a very pale green. Sections show that it is still at the undifferentiated meristematic stage, but the individual cells are considerably larger than those of the *Victoria plum* (Text-fig. 3, *a, b*). Both upper and lower epidermal cells bear large multicellular hairs, the heads of which are bent back along the surface of the leaf. The leaflets at the next stage, of which no diagram is given, have unrolled, they are of a very bright green, but are still very soft to touch. Sections now show a differentiation between the future palisade and spongy mesophyll; the former are typically brick-shaped, while the cells of the layer below have become more rounded off and intercellular spaces are beginning to appear. Nuclear divisions are not so frequent as in the mesophyll cells of the plum leaf, and



the resulting tissue consists of comparatively wide cells. There is, however, in the leaf of *Sambucus*, like that of *Victoria* plum, a difference in the rate of cell division in the upper mesophyll layer and those below, and the walls laid down in the former layer are at right angles to the surface.

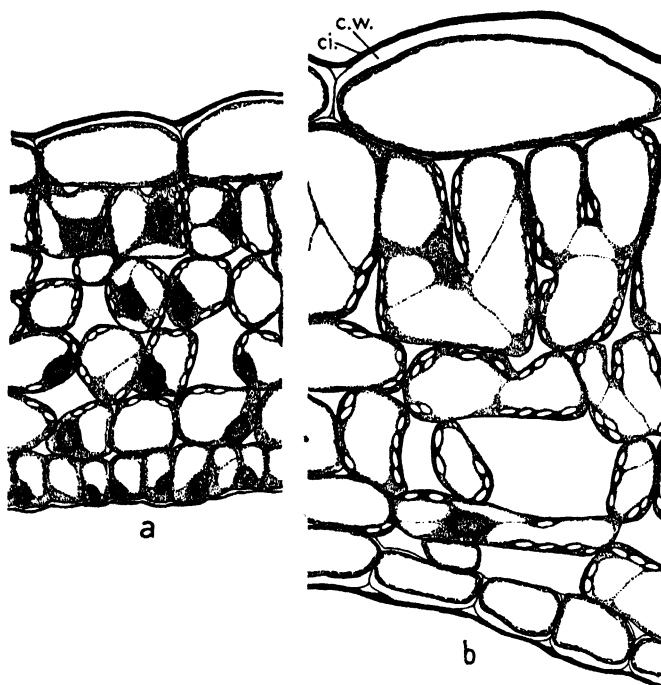


TEXT-FIG. 3. *Sambucus nigra*. a. Diagram of transverse section of leaflet, stage 1.  $\times 25$ . b. Transverse section of part of leaflet, stage 1.  $\times 350$ .

The cells of the upper epidermis at this stage are just beginning to stretch in the direction parallel to the leaf surface, but the cells of the palisade layer continue to divide and the two layers thus 'keep pace' with each other.

At stage 3 (Text-fig. 4, a), the leaflets look as if they had reached maturity, but they are still rather softer than fully matured leaves, and

they are of a very vivid green. It is at this stage that the first signs are visible of a change from typical palisade cells to 'arm' palisade cells. The cells of the upper epidermis have stretched enormously in a tangential direction (cf. Text-fig. 3, *b* and Text-fig. 4, *a*), and the rate of cell division



TEXT-FIG. 4. *Sambucus nigra*. *a*. Transverse section of part of leaflet, stage 3; leaflet vivid green,  $\times 350$ . *b*. Leaflet stage 4, showing mature 'arm' palisade cells; leaflet dark green,  $\times 350$ . c.w. = cellulose wall. c. = cuticle.

in the palisade is evidently not sufficiently high to maintain the brick shape of the palisade cells. Instead, the palisade cells have stretched with those of the upper epidermis, giving large, more or less square cells instead of the typical brick-shaped cells. Then the stretching stage is more marked in the wall adjacent to the epidermis than in the other walls of the palisade cells, for this wall becomes infolded towards the centre of the cell usually about mid-way along the wall. The stretching and rounding of the cells of the lower layers of the mesophyll give rise to the intercellular space system which is in communication with the external air by means of the stomata which have now completed their development. Cell division has now ceased throughout the leaf except in the vascular bundles which continue to enlarge for some time. The nuclei of the mesophyll cells of *Sambucus* are considerably larger than those in the leaf of *Victoria plum*, and they remain large until senescent changes begin to take place.

The transition from stage 3 to the mature stage is marked by a change from a very vivid green to a dark green together with a definite increase in the shininess of the upper surface. The increase in the tangential length of the upper epidermal cells has continued, while the cells of the mesophyll have increased very much in size. Those of the palisade are still nearly square, but they have increased in size slightly more in the direction at right angles to the surface than parallel with the surface, and the sides of the wall projecting into the cavity of the cell have become closed up and are now parallel to each other. The spongy mesophyll cells have increased very much in the same direction as that of the epidermal cells. These changes can be followed in Text-fig. 4, *a*, *b*, which were drawn from sections of fresh material.

The comparative thinness of the cuticle is an interesting feature of these leaves of *Sambucus*. The upper cellulose wall increases very much in thickness, but only a thin cuticle is deposited on the outer surface; this is clearly shown by sections stained with Sudan III in glycerine and alcohol.

The general development of *S. nigra* resembles that of the typical leaf in that cell differentiation takes place simultaneously throughout the leaf, but the duration of the phases of cell development in the mesophyll differs from that found in a typical leaf. Cell division in the mesophyll is not so rapid in *Sambucus*, and the cells forming this tissue are larger; the leaflets of *S. nigra* are generally narrower than the leaves of *Victoria plum*, but the width of the palisade cells is three or four times greater in the former leaf. Also the palisade cells of *S. nigra* are not so closely packed together as those of the plum, and are, more or less, square in cross-section. In both *S. nigra* and the plum leaf, cell division continues longer in the palisade layer than it does in the other mesophyll layers, but the stretching stage of the developing cells of *S. nigra* is more pronounced than in the typical leaf. Thus in the early stretching stages of *S. nigra* the palisade cells are more elongated in a tangential direction—a condition never shown by the leaf of *Victoria plum*—and the final stages are marked by the production of 'arm' palisade cells.

#### *PINUS SYLVESTRIS.*

Probably the best known example of a leaf with 'arm' parenchyma is that of *Pinus sylvestris* which is described in almost every text-book of botany. It is a needle-shaped, cylindrical leaf, and the mesophyll surrounds the central vascular tissue, and the development of the mesophyll makes an interesting comparison with that of other species of leaves which produce 'arm' parenchyma.

The leaves of *P. sylvestris* grow in pairs at the ends of shoots of limited growth. Early in the season the young leaves are covered with

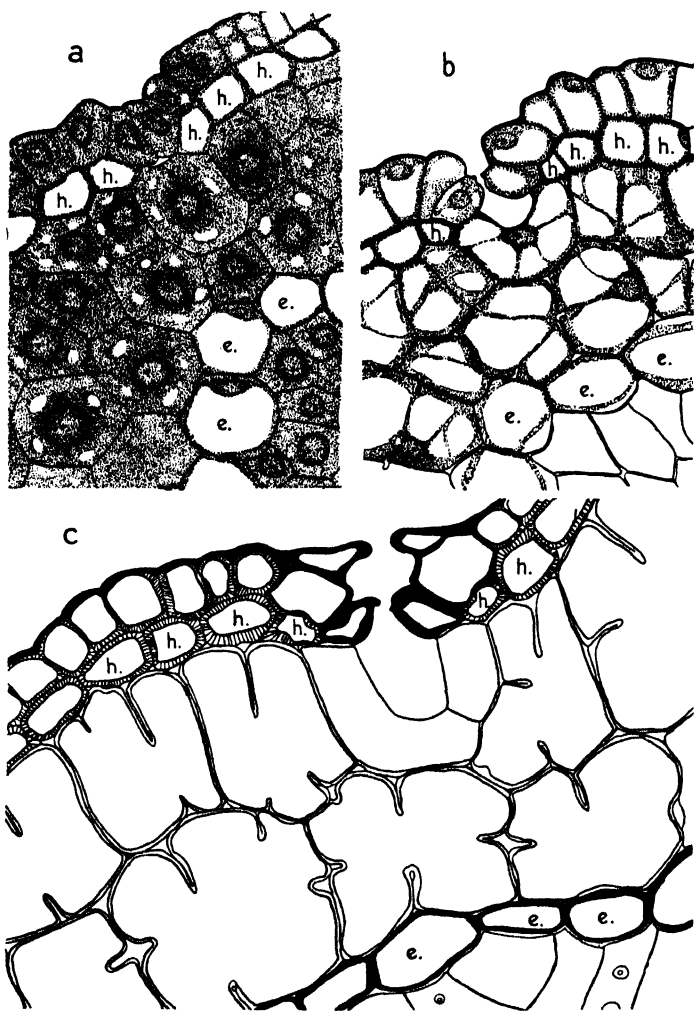
scales for several weeks during the first stages of development. Sections of the youngest leaves show that they consist of meristematic cells with extraordinarily little differentiation in size or shape throughout the leaf, with the exception of the two groups of xylem which can be distinguished near the centre of the leaf. The endodermis is differentiated next between the mesophyll and the central vascular tract, the latter formed by the vascular bundles surrounded by parenchyma cells with very thin walls which will later form the transfusion cells. The endodermal cells are vacuolated, but the walls are unthickened and the nuclei are still dividing, in fact, dividing nuclei can be seen in all the tissues at this stage. The mesophyll consists of large isodiametric cells, also very thin-walled, containing large nuclei (Text-fig. 5, *a*). The cells of the epidermis between the developing stomata are beginning to vacuolate as well as those of the hypodermis. The stomata already show the future guard cells above a minute sub-stomatal cavity.

The next stage (Text-fig. 5, *b*) is noticeable for the cessation of nuclear division in the mesophyll cells and the cells surrounding the vascular bundles. This coincides with the rapid development of the endodermis, which has now become a differentiated layer with the radial walls suberized on the outside. The epidermal and the hypodermal cells are also becoming thick-walled, and a cuticle of considerable thickness has been deposited on the outer wall of the epidermal cells. The vascular tissue within the endodermis has increased in area owing to the development of xylem and phloem, and the future transfusion cells have begun to vacuolate and to elongate radially; but at this stage they show no pits in their walls, which are still thin. The mesophyll cells have increased very much in size and have vacuolated, and the first signs of the infolding of the walls can be seen. The stomata are now fully formed, and the guard cells are slightly sunk owing to the continued growth of the cells on either side of them, and also to the enlargement of the sub-stomatal cavity.

At stage 3 (Text-fig. 5, *c*), the pair of leaves are no longer covered with scales, but they are still bright green in colour, and are growing rapidly in length. The walls of the cells of the epidermis, hypodermis, and endodermis have all increased markedly in thickness, and a thick cuticle is present on the outer wall of the epidermis. The 'arm' parenchyma cells have also increased in size, and show the typical folds on all their walls. This stage of growth and extension of the walls lasts for some considerable time, during which the folds in the cell wall project farther into the cavity of the cells, so increasing the wall area against which the chloroplasts lie. As in *S. nigra*, the walls of these cells have evidently a high stretching capacity, and in addition they are differentiated between two very thick-walled and relatively inextensible layers of cells—the hypodermis and endodermis—in which the thickening has commenced before

the extension stage has set in in the mesophyll cells. In this leaf the spatial arrangements of two specialized layers of cells play a very important part in the type of mesophyll cell formed in the mature leaf.

The stomata at stage 3 are very much sunk below the outer surface



TEXT-FIG. 5. *Pinus sylvestris*. Transverse sections of part of leaf. a. Stage 1. b. Stage 2. c. Stage 3.  $\times 350$ . h. = hypodermis. e. = endodermis.

of the leaf, and appear to have been formed in the hypodermis. The sub-stomatal cavity is large, and is formed—in many of the stomata—by the separation of the two segments of the mesophyll cell on either side of the ingrowth of the wall. This method of formation is seen in Text-fig. 5, b and c.

No diagram is given of a fully matured leaf as the microscopic appearance of a section through such a leaf is so familiar. The only differences between such a diagram and Text-fig. 3 would be that the cavity of the cells of the hypodermis and epidermis would be nearly obliterated owing to the increase in the thickness of the walls. This thickening goes on during the whole of the season, and continues for many months after the assimilating cells have reached their maximum size. Also, the parenchyma cells between the vascular bundles become thickened and form a central group of sclerenchyma.

The development of the leaf of *P. sylvestris* is exceptional in several ways, and it is interesting that these peculiarities in development can be correlated with the production of a specialized type of mesophyll. Throughout the length of the leaf run two parallel unbranched vascular bundles which are separated from the mesophyll by an endodermis. The cells of the epidermis, hypodermis, and endodermis mature quickly and become thick-walled at an early stage. The presence of a suberized endodermis at an early stage in the development of the leaf probably restricts the supply of food passing outwards to the mesophyll cells. At any rate the suberization of the cells of the endodermis is followed by the cessation of cell division in the mesophyll layers, and a change in the food supply to these layers may well be the determining factor in such a cessation of nuclear activity. The mesophyll cells are large and isodiametric at first, and cell division ceases early, but the walls have a high stretching capacity which is maintained for an abnormally long time; the cells develop rapidly between thick-walled layers of cells, thus producing cells with the infolded walls characteristic of 'arm' parenchyma.

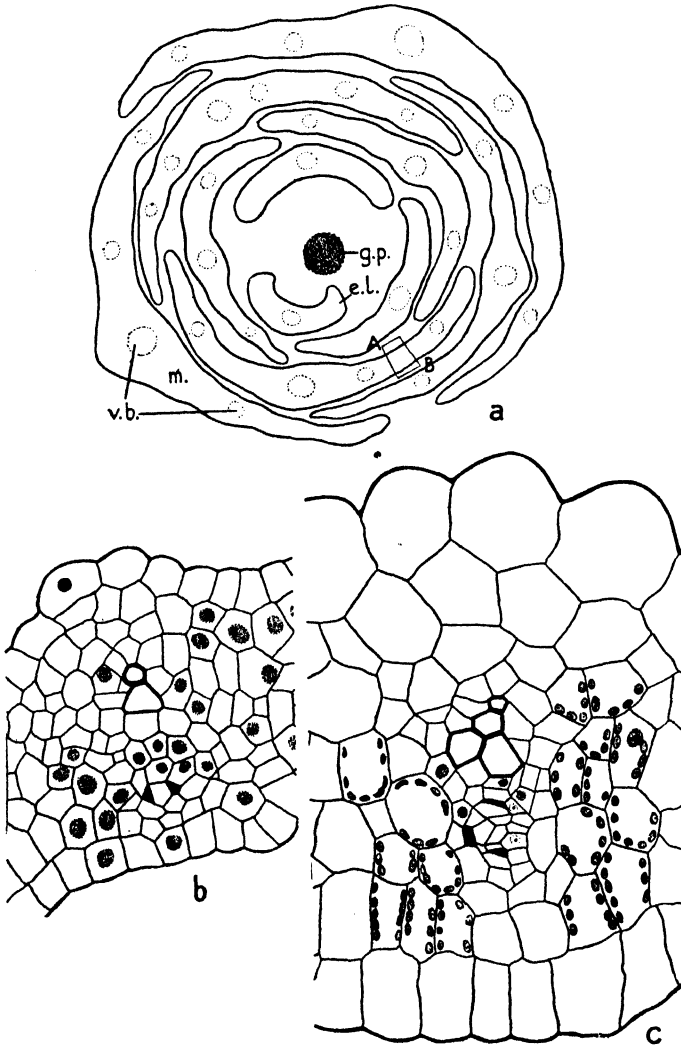
#### *ALSTROEMERIA AURANTIACA.*

The leaf of *Alstroemeria aurantiaca* is another example of a leaf in which 'arm' palisade tissue is formed, but what is of perhaps greater interest is that this layer is developed on, morphologically, the lower surface of the leaf which is brought uppermost in the mature leaf by the twisting of the petiole.

The leaves of *A. aurantiaca* are elongated, oval leaves with parallel venation. In the young stages they overlap each other closely at the stem apex, and are not protected in any way by bud scales. The development of the palisade layer on the lower surface of the leaf is thought to be connected with this fact, because, during their development, light will impinge most on the under surface of the leaf. This, however, does not seem to be a possible explanation, because the formation of typically shaped palisade cells occurs in the young leaves before they reach the outside of the bud. The leaves come away from the bud in an erect position, and it is some

time after this that the petiole becomes twisted and the lower surface comes uppermost; the blade is then held, more or less, in a horizontal position.

The growing point of the stem of this plant is an elongated cone of meristematic tissue and the embryonic leaf, when it becomes free from the



TEXT-FIG. 6. *Alstroemeria aurantiaca*. *a*. Transverse section of leaves in bud,  $\times 32$ . *b*. Transverse section of vascular bundle of leaf, stage 1,  $\times 310$ . *c*. Vascular bundle of leaf, stage 2,  $\times 310$ . v.b. = vascular bundle. m. = mesophyll tissue. g.p. = growing point of stem.

stem, consists of a comparatively broad band of meristematic cells with the developing vascular bundle in the centre (Text-fig. 6, *a* e.l.). The mesophyll at this stage consists of three rows of undifferentiated isodiametric cells with similar cells forming the upper and lower epidermis. The nuclei are

large in all the layers, and nuclear divisions are frequently to be found in the sections; the new walls are laid down at right angles to the surface of the leaf—thus adding to the width of the leaf without increasing the number of cell layers. The vascular strand consists of one or two protoxylem elements, and phloem elements are not distinguishable. The vascular bundles branch and the amount of mesophyll tissue increases, so that in transverse section the mesophyll consists of short bands of meristematic cells separated by the parallel vascular bundles (Text-fig. 6, *a*).

In the outer leaves of the bud, the number of layers of cells in the mesophyll is increasing to form four to five layers in the adult leaf. Pl. VIII, Fig. 1, which is a drawing of the part AB in Text-fig. 6 *a* of the sixth leaf beyond the growing point, shows the division of one of the mesophyll cells of the middle row by means of a wall parallel to the leaf surface; both the mesophyll and the epidermal cells are still meristematic at this stage, but the vascular supply is differentiating (Text-fig. 6, *b*). The development of the vascular supply, however, is very slow compared with that of a typical dorsiventral dicotyledonous leaf in which it is differentiated far in advance of the mesophyll, and in *A. aurantiaca* the bundles never attain the size of that of *Victoria* plum. In the adult *Alstroemeria* leaf the vascular bundles consist of four to five xylem elements and a very small group of phloem cells, both of which are surrounded by parenchyma, and the vascular tissue is developed slowly during the differentiation of the rest of the leaf, also, when once the bundle has reached a certain size no more conducting cells are differentiated (Text-fig. 6, *c*). As the mesophyll cells differentiate, the parallel vascular bundles become more separated and the mesophyll of *Alstroemeria* is not, therefore, differentiated in the meshes of a network of vascular tissue like that of *Victoria* plum.

The next stage in leaf development shown in Pl. VIII, Fig. 2, is a drawing of a small part of a leaf that has at least four more leaves beyond it and, therefore, covering it. It is clear that the differentiation of the mesophyll into typical palisade and spongy mesophyll has begun to take place. Nuclear divisions still continue in the cells of the palisade layer, and they still look meristematic, while the other mesophyll cells, although a few nuclear divisions can still be found, are vacuolating and enlarging rapidly, forming intercellular spaces at the corners of the cells. It is at this stage that the stomata are being differentiated on the, morphologically, upper surface (i.e. xylem surface). The cells of both the epidermal layers are vacuolating like those of the spongy mesophyll.

In the mature leaf shown in Pl. VIII, Fig. 3, great changes have taken place since stage 2 has been passed through. All the cells have stretched enormously; those of the two epidermal layers and the spongy mesophyll cells have stretched, especially in the direction parallel to the surface of the leaf, while the palisade cells have become more or less square in outline



with infoldings of the upper wall towards the centre of the cell. A thin cuticle is present on the outer surface of the walls of the 'upper' epidermal cells and a large intercellular space system has been developed. It will be seen that the amount of cell readjustment in this leaf is enormous.

The cell walls of the leaves of *A. aurantiaca* have also a very high stretching capacity. The palisade cells obviously have not so much stretching space as the spongy mesophyll cells, as they are more numerous and more closely packed than those of the spongy mesophyll. If the walls of the cells of the palisade layer have the same stretching capacity as those of the spongy mesophyll cells, then the increased wall area in the palisade layers at the stretching stage can only be accommodated by the infolding of the wall. Comparing this leaf with another monocotyledonous leaf—*Zea japonica*—with an undifferentiated mesophyll consisting of large isodiametric cells, it is possible, in *A. aurantiaca*, to correlate the differentiated mesophyll (i.e. into palisade and spongy mesophyll) coupled with abnormally high stretching capacity of the cell walls with the formation of 'arm' palisade in this leaf.

The theory that the formation of a palisade layer on the morphologically lower surface of this leaf is due to the development of the bud in the light and hence to the action of light on the cells of this leaf surface received no support, unless light impinging on the outside of the bud can be effective in this way through many layers of cells, for the mesophyll is differentiated long before the leaf reaches the outside of the bud.

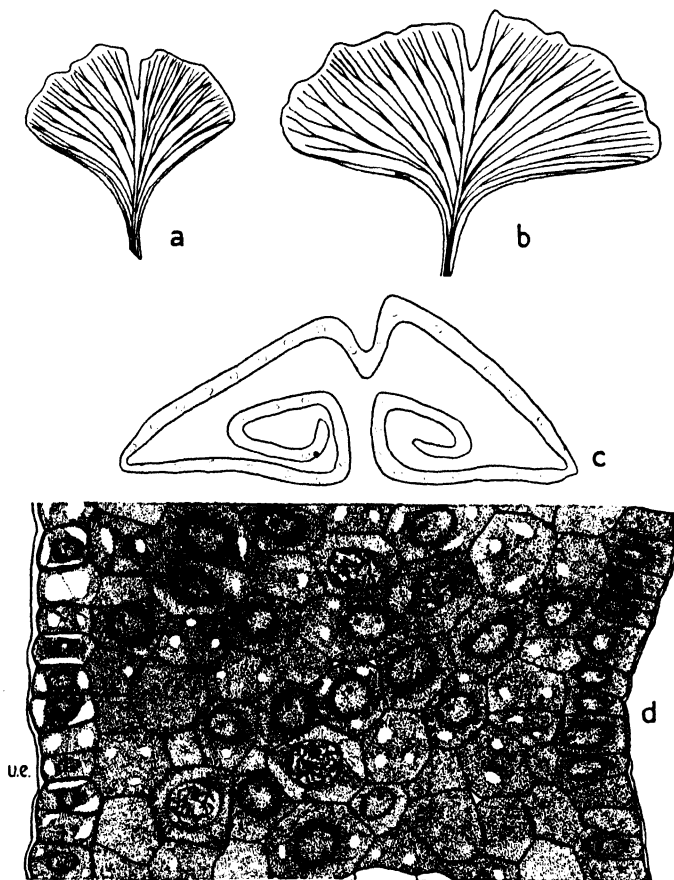
#### *GINKGO BILOBA.*

*Ginkgo biloba* has the oldest fossil record of any of our living plants, and it has leaves of a peculiar shape which are a complete contrast to that of any of the previous examples.

When the leaf emerges from the bud, the two lobes of the leaf are folded in towards each other (Text-fig. 7, *c*), but it becomes flattened out later and the lateral margins of the leaf then make an angle of about  $135^\circ$  with the petiole (Text-fig. 7, *a*). Two vascular bundles enter at the base of the leaf and the whole of the vascular tissue in the leaf is supplied by the subsequent dichotomy of these two bundles and their branches. In the early stages the leaf is a pale yellow-green, but it becomes a typical dark green about half way through its development. During this colour change, it becomes more fan-shaped owing to the continued development at the margin of the leaf, and both lobes of the leaf increase in area; the angle formed by the petiole and the lateral margins becomes less, so that in a mature leaf this angle is about  $90^\circ$  (cf. Text-fig. 7, *a* and *b*).

Sections of the leaves while still folded in the bud show that the cells of the leaf are all meristematic except those of the developing vascular

bundle. The cells of the mesophyll are more or less isodiametric in shape; they are large and have large nuclei which show the various stages of nuclear division very clearly. The upper epidermal cells are beginning to

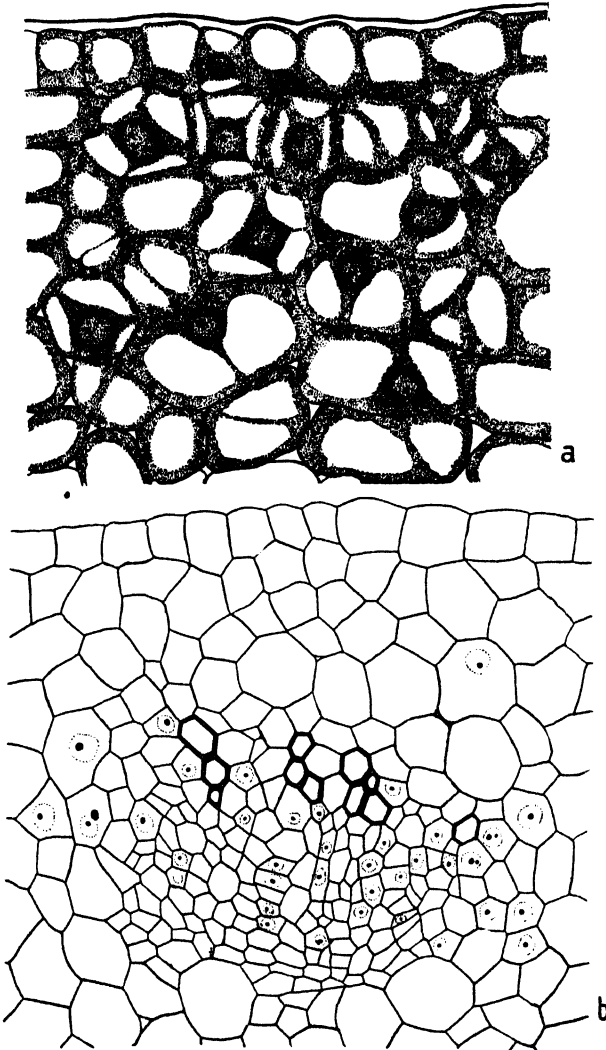


TEXT-FIG. 7. *Ginkgo biloba*. a. Young leaf. b. Mature leaf (both half natural size). c. Transverse section of leaf in bud,  $\times 8$ . d. Transverse section of leaf, stage 1,  $\times 310$ . u.e. = upper epidermis.

vacuolate, but those of the lower epidermis are still dividing, but no stomata have yet been formed (Text-fig. 7, d).

When the leaf is beginning to flatten out, marked changes in the shape of the cells of the mesophyll are to be seen. In spite of active cell division, which is such a feature of sections of leaves at the earliest stage, the cells have increased in size very much in the direction parallel to the surface of the leaf; more cell divisions have, however, taken place in the uppermost mesophyll layer, and the cells so formed are more or less square in shape with no intercellular spaces between them (Text-fig. 8, a). Nuclear divisions

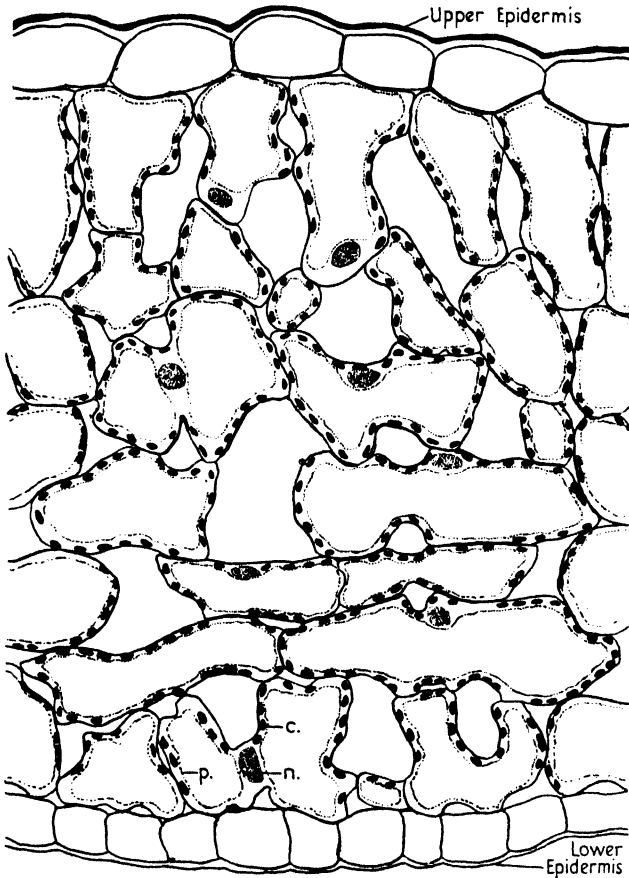
cease sooner in the lower mesophyll layers than in the upper layer, and the cells of the former layers have begun to elongate in the direction parallel to the epidermis. The vascular bundles develop very slowly and, like those



TEXT-FIG. 8. *Ginkgo biloba*. *a*. Transverse section of part of leaf, stage 2,  $\times 310$ . *b*. Transverse section of vascular bundle of leaf, stage 2,  $\times 310$ .

of *Alstroemeria*, separate the active mesophyll cells up into groups of developing cells, so that, when these cells begin to extend, the vascular bundles merely become more separated. A diagram is given in Text-fig. 8, *b* of a vascular bundle drawn from a leaf at stage 2; xylem elements have been differentiated, but no thick-walled parenchyma cells are developed as

in Victoria plum, and the bundle is embedded in the assimilating tissue. The most resistant cell layers are those of the epidermis and the margin. These, however, are differentiating only slightly ahead of the mesophyll



TEXT-FIG. 9. *Ginkgo biloba*. Transverse section of part of leaf, stage 3,  $\times 310$ . n. = nucleus. c. = chloroplast. p. = protoplast.

cells and consequently the mesophyll cells can extend with the epidermal cells until the latter have reached their mature size.

At stage 3 (Text-fig. 9) the growth and stretching of the cells have continued, producing palisade cells elongated at right angles to the epidermis, but very much broader than those of typical dorsiventral leaves especially where they are contiguous with the epidermal cells. Large intercellular spaces are present between the long walls of this layer and throughout the rest of the mesophyll. The latter consists of very elongated cells some of which are shown in Text-fig. 9, although, at this stage, they are frequently even more elongated than those in the drawing. The layer next to the

lower epidermis is generally formed by very lobed cells as if the cells of this layer have not been able to stretch completely in the direction parallel to the epidermis owing to the close connexion between the cells at certain points.

After the cells have reached their mature size, there is a marked increase in the thickness of the cuticle, especially that on the upper surface of the leaf. The stomata are formed only in the lower epidermis, and after their formation they become sunk owing to the continued division of the epidermal cells on either side of them.

The characteristic features of the development of the leaf of *Ginkgo biloba* can be summarized as follows:—1. The development of the leaf is definitely marginal, the youngest part of the leaf being that just within the lateral margin. 2. The cells of the apical margin and the epidermal cells vacuolate and stretch only very slightly in advance of the mesophyll cells. This process takes place at the same rate in both tissues, hence the cells forming each sector between the arms of the dichotomy of a vascular bundle grow as a unit. The result of this growth is a widening of the angle between the arms of the dichotomy of a vascular bundle, and consequently it exerts little restraining influence on the stretching stages of the internal tissues. 3. The mesophyll is differentiated into palisade and spongy mesophyll, but the lack of pressure on the developing cells is reflected in the fact that the palisade cells are broad and the spongy mesophyll cells are very elongated in the direction parallel to the surface.

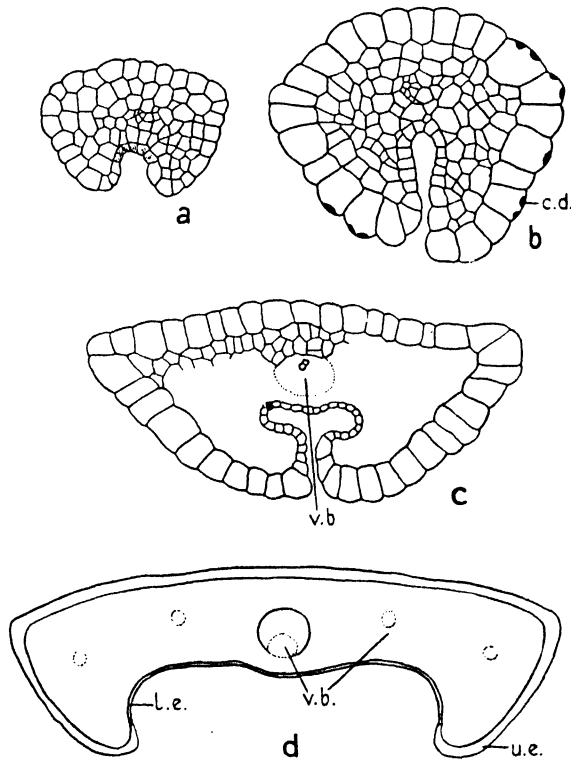
#### *ERICA BACCANS.*

The leaves of the genus *Erica* are narrow and elongated and the margin is rolled back against the lower surface. This type of leaf is so characteristic of this genus that when it is found in other genera it is usually called an ericoid leaf. The development of the leaves of six species of *Erica* was worked out (*Erica albens*, *E. baccans*, *E. glandulosa*, *E. maritima*, *E. perspicua*, *E. ramulosa*), but every example showed the same features, consequently the description of the development of the leaf of *E. baccans* will cover that of the other species.

The youngest stage in leaf development is that in which the embryonic leaf consists of an erect cone of meristematic cells at the base of which, in transverse section, the procambial strand can be distinguished as a group of very minute cells. This undifferentiated stage is of very short duration and it is followed by the vacuolation and enlargement of the upper epidermal cells which takes place at an extraordinarily rapid rate and is the dominating feature of the next two stages. A transverse section through a leaf at this stage shows that the epidermal cells are the only cells to have enlarged to any extent since the meristematic stage. The vascular supply is still only a procambial strand and the mesophyll cells are small and meri-

stematic and are dividing, thus adding to the number of the cells without any appreciable increase in cell size. The lower epidermal cells are small and square and there are very few of them compared with those of the upper epidermis (Text-fig. 10, *a*).

The rapid increase in size of the upper epidermal cells is a noticeable



TEXT-FIG. 10. *Erica baccans*. Transverse sections of leaf. *a*. Stage 1. *b*. Stage 2. *c*. Stage 3. *d*. Plan of transverse section of mature leaf. *a-c*,  $\times 250$ . *d*,  $\times 45$ . v.b. = vascular bundle. u.e. = upper epidermis. l.e. = lower epidermis. c.d. = cellulose deposit.

feature throughout the early stages of development. They undergo division, and owing to the extension of the cell walls the wings of mesophyll cells on either side of the main vascular bundle almost meet below the bundle. The lower epidermal cells do not increase much in number or size for some time, consequently they do not compensate in any way for the very rapid development of the cells of the upper epidermis. Also, very little alteration can be seen in the cells of the mesophyll; they increase in number but remain small, isodiametric, and have dense contents. The vascular bundle, too, remains practically undifferentiated, although in some sections a xylem vessel can be distinguished, but at this stage the strand is still unbranched.

At stage 2, the lower epidermal cells have divided and have increased

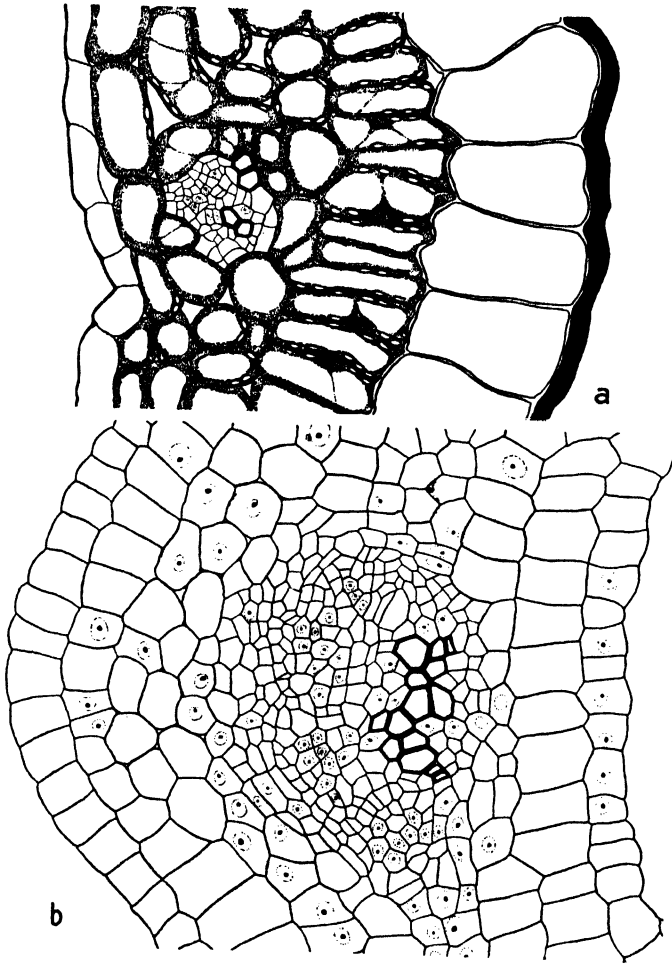
in number, but they are still small and full of contents and there are no signs of stomata. The upper epidermal cells are continuing their development and still dominate the sections as regards size. They show signs of a very active cellulose metabolism at this stage, for in this species deposits of cellulose forming small lumps on the wall can be seen in transverse section (Text-fig. 10, *b*); these deposits disappear at the next stage, presumably owing to their absorption into the fabric of the wall. The mesophyll cells are now beginning to show signs of vacuolation, but there is only a very slight indication of any differentiation into the palisade and spongy mesophyll which is present in the adult leaf. The vascular supply is equally slow in its differentiation, one or two xylem elements can be distinguished and some phloem, but the bundle is beginning to be separated off from the mesophyll cells by a ring of more vacuolated cells which later will form the vascular sheath. The development of the vascular supply of *Erica baccans* is in several ways unlike that of a typical dorsiventral leaf. It is not, as in *Victoria plum*, the first tissue to be differentiated to any extent, and its development is not rapid; also, it is completely embedded in the mesophyll tissue and only separated from it by a vacuolated layer when the leaf has undergone a good deal of development (Text-fig. 11, *a*).

At the next stage in the development of the leaf, the upper epidermal cells have become more elongated in the direction parallel to the leaf surface and the leaf consequently has flattened out along the upper surface and has become wider in transverse section, but it is still very rolled (Text-fig. 10, *c*). The vascular supply shows considerably more differentiation in the central vascular bundle and the two branch bundles can also be distinguished. The mesophyll cells are more vacuolated and the upper layers are beginning to elongate in an anticlinal direction. There is still no sign of the formation of any intercellular space system and no stomata are present in the lower epidermis which still consists of very small cells.

With the continued differentiation of the vascular supply and the development of more branches, the differentiation of the mesophyll into palisade and spongy mesophyll becomes more rapid. At the same time the stomata are formed in the lower epidermis and the intercellular space system develops simultaneously. This final stage in the differentiation of the mesophyll takes place rapidly and the differentiating cells undergo considerable extension (Text-fig. 11, *a*). After the differentiation of the mesophyll the leaf becomes less rolled as the surface flattens out with the extension of the cells forming the interior of the leaf, and the leaf is now rolled only at the outer margin (Text-fig. 10, *d*). The upper epidermal cells have become more thick-walled and a considerable cuticle has been deposited gradually. The walls of the cells forming the vascular sheath have also increased in thickness.

The development of these ericoid leaves was compared with that of a member of the Ericaceae—*Macleania punctata*—which has a flat leaf. Two

differences in the development of the tissues of this leaf were of interest. The upper epidermal cells of *M. punctata* differentiate before those of the lower epidermis; differentiation proceeds at a normal rate as in *Victoria plum* and takes place only slightly ahead of the mesophyll cells within and



TEXT-FIG. 11. a. *Erica baccans*. Part of differentiated leaf showing vascular supply,  $\times 450$ . b. *Macklania punctata*. Vascular supply of meristematic leaf,  $\times 310$ .

produces no rolling of the leaf, and it is differentiated in conjunction with a hypodermal layer. The vascular supply of this leaf is differentiated more like that of a normal dorsiventral leaf than that of an ericoid leaf. It is differentiated more rapidly, and consequently is of some considerable size in the young stages of the leaf. A comparison of Text-figs. 11, a and b will show this point; the diagram of the vascular bundle of *M. punctata* is that



of a young leaf in which the mesophyll is still meristematic, while that of *E. baccans* is the vascular bundle in a mature leaf. The comparison of the development of the leaves of *E. baccans* and *M. punctata* suggests that the ericoid type of leaf can be correlated with the rapid development of the epidermal layer coupled with the very slow differentiation of the vascular supply.

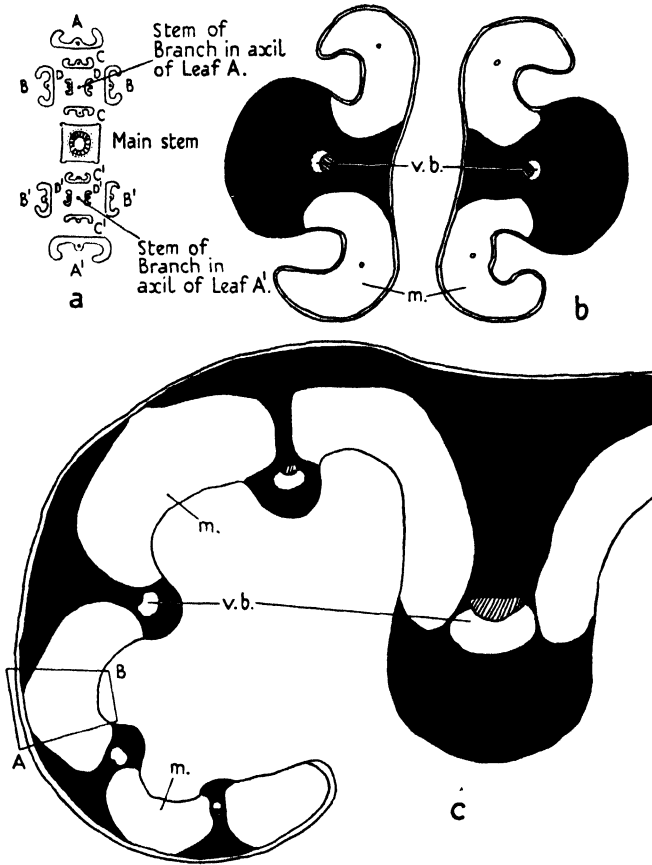
The duration of the various phases of development in the tissues of ericoid leaves is of interest. The epidermal cells in ericoid leaves pass through a very short meristematic stage which is succeeded by an abnormally long vacuolating and stretching stage. In the early vacuolating stages nuclear divisions still continue, but protein metabolism is evidently very soon replaced by carbohydrate metabolism when cellulose is formed in excess of the immediate requirements of the leaf. After the phase of cell extension has been passed through, the main trend in metabolism in the cells of this layer appears to be that of fats which produces the comparatively thick and inextensible cuticle of the mature leaf. In the mesophyll cells and those of the lower epidermis, on the other hand, the meristematic stage is the dominant one and lasts far longer than the succeeding stage, which is passed through very rapidly. On the basis of cytological data, it seems probable that the raw materials for carbohydrate synthesis are, more or less, monopolized by the upper epidermal cells for some considerable time. Meanwhile protein synthesis is carried on to a certain extent by the mesophyll cells, thus adding to the bulk of this tissue, but delaying its differentiation. With the completion of most of the stretching stage in the upper epidermal cells, more raw materials are set free for the mesophyll layers and lower epidermal cells which pass rapidly through the last differentiation stages and form the typical palisade and spongy mesophyll layers of the mature leaf.

#### *ROSMARINUS OFFICINALIS.*

The leaves of *Rosmarinus officinalis* are borne in groups in the axils of two opposite leaves (formed in the previous season) on the main stem. Each group consists of about three pairs of leaves borne very close together on a shoot of limited growth. A diagram showing the arrangement of the pairs of leaves is shown in Text-fig. 12, *a*. Sections through each pair of these leaves (A, B, C, and D) show every stage in the development of the leaf.

The youngest pair of leaves (D) lie flat against each other; they are white in colour and very soft to touch. Sections through these leaves show that they consist principally of a large main vein, on either side of which are two wings of mesophyll tissue surrounded by a meristematic epidermis (Text-fig. 12, *b*). The majority of the epidermal cells have given rise to projecting multicellular hairs, to the presence of which the whiteness of these

very young leaves is due (Pl. VIII, Fig. 4). The branch bundles are just beginning to develop in the outer part of the mesophyll; they are derived from the main vein, but can only be distinguished by the presence of a

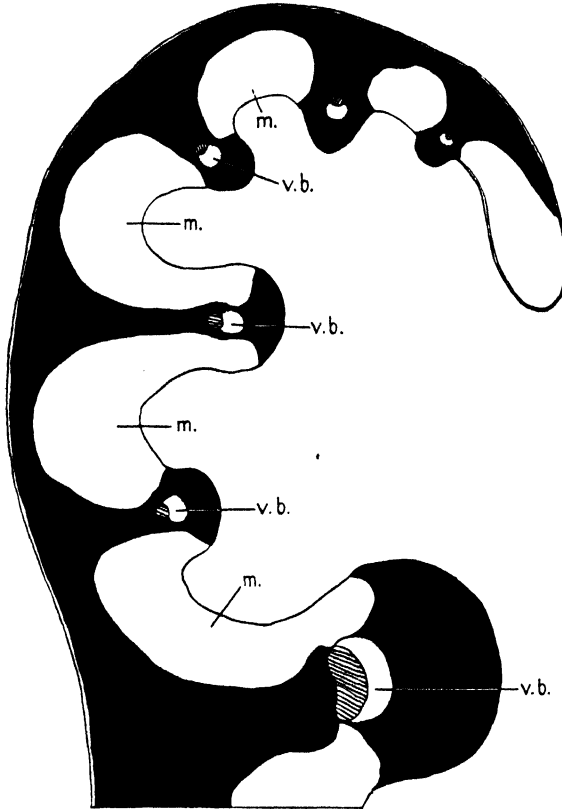


TEXT-FIG. 12. *Rosmarinus officinalis*. a. Plan showing arrangement of leaves of branch. b. Diagram of leaf D showing distribution of tissues,  $\times 25$ . c. Diagram of leaf C showing distribution of tissues,  $\times 25$ . Solid black = thick-walled accessory tissue. m. = mesophyll. v.b. = vascular bundle.

group of minute cells which are slightly differentiated and which will later form the xylem elements.

The two next older pair of leaves (C) show a very much more complicated structure. The vascular tissue now consists of four parallel bundles on either side of the main vein (Text-fig. 12, c). These branch bundles are connected with each other by means of small cross bundles. The leaf is now very definitely rolled, the margin of the leaf nearly touching the large main vein, and the lower surface is grooved to a small extent. The lower epidermal cells are still meristematic, and some of these show the early

stages of stomata formation ; innumerable long branched hairs, as well as short glandular hairs project from this surface. The upper epidermal cells have enlarged and have become differentiated except for a few cells midway between the vascular bundles (Pl. VIII, Fig. 5). Below the upper epidermis



TEXT-FIG. 13. *Rosmarinus officinalis*. Diagram showing distribution of tissues of leaf A (Text-fig. 12),  $\times 8$ . Solid black = thick-walled accessory tissue. v.b. = vascular bundle. m. = mesophyll.

and extending from that layer to the vascular supply, bands of thick-walled cells have been differentiated which have not yet joined up to form a continuous layer below the epidermis (Text-fig. 12, c). At this stage the palisade layer consists of typical elongated cells which are still meristematic in appearance and in which chloroplasts can just be distinguished. The intercellular space system is beginning to be formed in connexion with the development of the stomata in the lower epidermis. The hairs on the upper epidermis have shrivelled and have disappeared, and a cuticle has been deposited on the upper wall of these cells.

As the vascular bundles become differentiated, the cells forming the thick-walled tissue above them become more elongated at right angles to

the epidermis, and they develop conspicuous pits in their walls. This elongation results in a large increase in the width of the bundle region between one surface of the leaf and the other: this can be seen clearly in Text-fig. 12, *c* and Text-fig. 13 in which the former is a diagram of leaf C at a magnification of 25 and Text-fig. 13 is leaf B, but at a magnification of 8. The second vascular bundle from the main vein in leaf B shows the greatest increase in depth, but all the others have increased proportionately. The lower epidermal cells have continued to divide while the blocks of thick-walled cells have been developing, with the result that deep grooves have been formed along the lower surface of the leaf. These blocks of thick-walled cells are differentiated first above each vascular bundle and differentiation then continues on either side of the bundle until the blocks of cells meet each other halfway between the vascular bundles. In this way they form arches of thick-walled cells above the developing mesophyll cells. The differentiation of the upper epidermal cells follows that of the blocks of accessory tissue, and they remain meristematic for some time in the centre between two vascular bundles (Text-fig. 12, *c*).

The mature mesophyll cells show the usual differentiation into typical palisade and spongy mesophyll cells. In leaf D (Pl. VIII, Fig. 4) it consists of three or four layers of meristematic cells all more or less palisade in shape with no intercellular space system yet developed. The differentiation between palisade and spongy mesophyll is noticeable in leaf C (Pl. VIII, Fig. 5), where the two upper layers of mesophyll cells are palisade in shape (they still look meristematic, and nuclear divisions are to be seen) and the cells of the lower layers have begun to enlarge and vacuolate, and the nuclear divisions are of less frequent occurrence. Intercellular spaces between the latter cells can now be seen, and early stages in the formation of the stomata in the lower epidermis are to be found at this stage. Complete differentiation of the mesophyll has taken place by the time the leaf has reached the stage of leaf B, and all the cells are fully vacuolated and a large intercellular space system has been developed in the spongy mesophyll in connexion with the stomata.

The vascular supply is differentiated very early in the development of this leaf, like that of typical dorsiventral leaves. The main vascular bundle in leaf D, for example, consists of eight rows of xylem, below which is a large development of vascular parenchyma; leaf C shows twelve to fourteen rows of xylem, and the cells forming the parenchyma have enlarged considerably and have become thick-walled. The branch veins develop in the same way, but they are considerably smaller in size and usually only five or six rows of xylem are formed and comparatively little parenchyma on the lower surface.

The development of the grooved leaf of *Rosmarinus officinalis* can be correlated with the formation of a thick-walled accessory parenchyma tissue

which originates above each vascular bundle and finally overarches the mesophyll tissue. The upper epidermal cells remain meristematic for a longer time than those of a typical dorsiventral leaf and only become differentiated when the arches of accessory parenchyma are complete. Otherwise, the development of the mesophyll cells and the lower epidermis is typical of that of flat dorsiventral leaves.

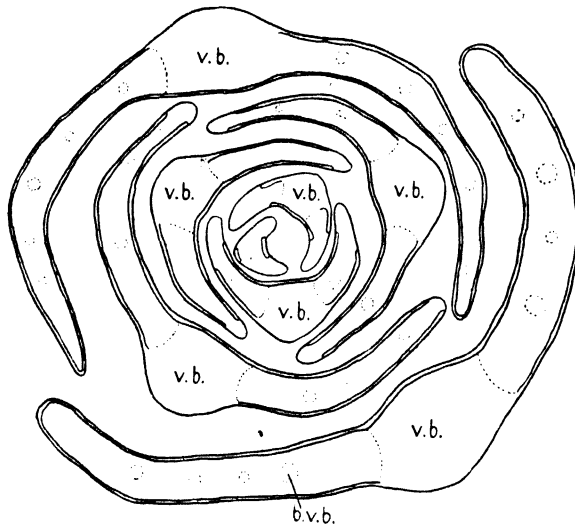
*SALIX ALBA.*

The leaves of this tree develop very rapidly when once they have emerged from the bud, and they complete their development in a comparatively short space of time. The buds are very elongated just before they begin to open, and the narrow linear leaves are folded one inside the other before the leaves become separated from each other (Text-fig. 14, *a*). Sections through buds at this stage show that a large amount of differentiation of the leaf tissues has already taken place. The innermost leaf has a vascular bundle in which two or three xylem elements have been differentiated, and some vacuolated cells are present above and below the bundle, otherwise the rest of the cells are meristematic (Pl. IX, Fig. 6). The outer leaves already show differentiation in the mesophyll, but not into the typical palisade and spongy tissue which is not formed in this leaf. In *Salix alba* the layer of cells forming the mesophyll layer within the epidermis vacuolate and elongate very early in the development of the leaf, producing a layer which looks like many hypoderms in some mature leaves. This layer of very vacuolated palisade shaped cells consequently is very conspicuous in the young leaves between the meristematic epidermis and inner mesophyll cells (Pl. IX, Fig. 7). The latter cells are also at this stage mostly palisade shaped in transverse section, and nuclear divisions are of frequent occurrence. Text-fig. 14 shows the gradual extension of the vacuolated mesophyll layer on either side of the main vascular bundle until, in the outer leaves of the bud, it forms a complete layer round the inner mesophyll layers, all of which, at this stage, are palisade in shape.

The vascular supply in this leaf is differentiated very rapidly, which perhaps is not surprising considering the astonishingly rapid growth of the leaves in early spring. The main vascular bundle consists of several rows of xylem, even in the inner leaves of the bud, and phloem is recognizable later when leaf extension is nearly complete.

During the early meristematic stages the leaf becomes broader owing to the cell divisions in all layers of cells, especially those beyond the vacuolated mesophyll layers; this broadening of the leaf is coincident with the branching of the vascular tissue which carries up the food supply for the cells of the mesophyll before carbon assimilation has begun. After this stage of development has been passed through, all the mesophyll cells elongate at right angles to the leaf surface, forming several layers of palisade

shaped cells (Pl. IX, Fig. 7). There is evidently no difference in the rate of, or in the length of, the duration of cell division in the various mesophyll layers, consequently a uniform tissue is produced with intercellular spaces distributed evenly throughout; the intercellular space system, however, is



TEXT-FIG. 14. *Salix alba*. Transverse section of leaves in bud—inner line shows extent of vacuolated mesophyll cells,  $\times 32$ . v.b. = main vascular supply of leaf. Broken line indicates branch vascular bundle.

not large compared with that of dorsiventral leaves in which it is mainly developed in the spongy mesophyll layers. In the mature leaves of *S. alba* the cells of the outer mesophyll layer are very like those of the equally vacuolated inner mesophyll layers and show no sign of having been differentiated earlier than the other layers (Pl. IX, Fig. 8).

The differentiation of the epidermis in this leaf is very interesting. The epidermal cells on both surfaces of the leaf remain meristematic for an abnormally long time, as if the vacuolated mesophyll cells below were acting in the capacity of a vacuolated epidermis, for the former cells differentiate far more like the epidermis of a normal dorsiventral leaf than like a mesophyll layer. Also, the lower epidermal cells tend to vacuolate and to differentiate before those of the upper epidermis, in fact, the last epidermal cells to differentiate are those of the stomata formed in the upper epidermis. Numerous stomata are formed on both surfaces of the leaf, but many of those on the upper surface are not formed until the leaf has practically completed its differentiation. Pl. VIII, fig. 3 is a drawing of a leaf in which the mesophyll cells are fully differentiated, but a stoma in a very early stage of development is present in the upper epidermis, while a mature stoma can be seen in the lower epidermis.

The leaf *S. alba* is an example of a leaf in which the mesophyll is not differentiated into palisade and spongy mesophyll. Nuclear divisions take place more or less uniformly throughout all the mesophyll cells, and the vacuolating and stretching stages are initiated at the same time in all the layers, except in the cells of the outer mesophyll layer which vacuolate and stretch considerably in advance of the mesophyll cells within.

The development of the tissues of this leaf suggests that in it there is a very different cycle of metabolism going on compared with that of a typical dorsiventral leaf, and the following is a tentative suggestion based on cytological evidence only. Protein metabolism characteristic of meristematic cells is maintained longer in the epidermal cells than in any other tissue, while carbohydrate metabolism is very soon established in the layer of mesophyll cells below. But in the inner mesophyll cells protein metabolism is dominant for some time longer than in the outer mesophyll layer; cell extension follows (indicating the beginning of carbohydrate metabolism) and nuclear divisions continue until carbohydrate metabolism has been established, after which cell wall extension is completed in these layers. Protein metabolism continues in certain groups of cells of the upper and lower epidermis; these eventually form the stomata on the surface of the leaf, and many of them (especially in the upper surface) are differentiated only when the rest of the leaf tissues are almost mature.

#### DISCUSSION.

The examples given of some of the methods of development to be found in the foliage leaves of Angiosperms and Gymnosperms illustrate the diversity that exists in the development of the mature leaf. Although the same tissues are present in all the leaves described—with the exception of *Pinus sylvestris* and *Rosmarinus officinalis* in which additional tissues go to make up the mature leaf—there is a marked difference in the rate of, and also in the extent of, the development of the tissues. During their development these tissues all make a different demand upon the available food supply, and the relationship between supply and demand in the tissues forms the basis of a metabolic system, the method of working of which must be of fundamental importance in the development of the organ as a whole. Bernard Dürken, in his 'Experimental Analysis of Development' (2), stresses the importance of trying to build up a picture of the processes going on in an organism as a whole; he says: 'We have in the processes of development what is by far the most interesting manifestation of life, and what is, at the same time, of the utmost importance for our understanding of the organism. No other function of living things characterizes life so exactly as development.... The experimental study of development embraces a formal and a causal statement of its problems, and its

general aim must be to connect all the single results obtained so as to form a coherent picture of what happens in development and thus to reveal the essential nature of its processes. . . . Development does not simply consist of a sum of independent processes running side by side, but is one undivided activity in which all processes and all the parts of the germ are closely knit. These interactions during development constitute its most essential characteristics'. Unfortunately, with our present technique only a meagre idea of the interactions and workings of the parts of this system can be obtained. One can, however, determine approximately which phases of development are the most dominant in the various leaf tissues and so gain some idea of the fundamental processes on which the development of the tissues of the mature leaf depend.

As an approximation, it is probably safe to assume that nuclear division is an indication of the dominance of protein metabolism and that the vacuolation of the protoplast is the first visible sign of a change over to carbohydrate metabolism. Lastly, when the cessation in the extension of the cell wall occurs, the cell can be regarded as having reached the maturing stages of development and the walls tend to become inflexible. These three phases of growth are cytologically distinguishable in stained sections of fixed material, and a study of these sections has shown that they may last for very varied times in different tissues of the same leaf and in the corresponding tissues of different species of leaves.

In the earliest meristematic stages, all the cells of the embryonic leaf are dividing rapidly, and during this time the total amount of protoplasm is increasing, suggesting that, at this stage at any rate, the metabolism is the same in all the cells. This meristematic activity throughout the whole leaf is of short duration. The vascular supply is the first tissue to be differentiated and, in a typical dorsiventral leaf, this is followed by that of the epidermal cells immediately above and below.

The development of the vascular bundle is a continuous process and may last for some time. The xylem is laid down first and continues to be added to while the leaf is growing in length, and the phloem is only distinguishable later in the development of the bundle. The examples described in this paper show that the final size of the mature vascular bundle varies very much in the different species, and it may be developed in connexion with accessory parenchyma as in *Rosmarinus officinalis*. In this leaf the formation of a thick-walled accessory parenchyma tissue developed especially in connexion with the main vascular bundles was found to be the main contributory cause of the grooving of the leaf. The development of this tissue results in a large increase in the depth of the leaf in the region of the vascular bundles; arches of thick-walled cells are formed, in the curve of which the mesophyll is differentiated into typical palisade and spongy tissue. The size and the rate of differentiation of the



vascular bundle in some examples may exert a considerable influence on the type of mature mesophyll cell produced in the leaf, and the shape of the palisade cells in a typical dorsiventral leaf can be regarded as an indication of the influence of the vascular supply on the stretching stages of the cells developing in close contact with it. In the parallel-veined leaf of *Alstroemeria aurantiaca* the vascular bundles are not so extensive and they complete their development soon, and are typical of the closed vascular bundles of most monocotyledons. Moreover, they are differentiated simultaneously with and not ahead of the mesophyll cells. Consequently the stretching stages of the mesophyll cells are not influenced in the same way as in net-veined leaves and the individual cells can stretch more easily in the direction parallel to the surface of the leaf. This is more emphasized in the development of the leaf of *Ginkgo biloba* in which the palisade cells are square in transverse section and the spongy mesophyll cells are very elongated in the periclinal direction. In this leaf the vascular supply develops with the mesophyll tissue and the epidermal and marginal cells.

The mesophyll, in its youngest stages, is completely dependent on the supply of food obtainable from the vascular supply. This condition lasts until the chloroplasts are formed. The functioning of the chloroplasts represents a big change over of the direction of metabolic activity, about which we know very little. The mesophyll of a typical dorsiventral leaf is differentiated into an upper layer of palisade cells elongated at right angles to the leaf surface and lower layers of irregularly shaped spongy mesophyll cells between which the intercellular space system is developed. This differentiation can be correlated with a difference in the length of duration of the three phases of development in the palisade and spongy mesophyll. Nuclear divisions take place more frequently and continue for a longer time in the palisade layers than in the cells of the spongy mesophyll. They take place throughout the early vacuolation stages of the palisade cells, indicating that the metabolism of protein and carbohydrates are then going on simultaneously, but with the development of chloroplasts the latter gradually becomes dominant and the nuclear divisions cease. The early and marked development of the vascular supply coupled with a high rate of nuclear division in the palisade layer are correlated with the formation of the typical shaped palisade cell. Another type of mesophyll development is illustrated by the 'arm' palisade of *Sambucus nigra* and *A. aurantiaca* in which differentiation of the mesophyll into palisade and spongy tissue takes place, but in both these examples the nuclei of the palisade layers do not continue to divide to the extent found in a typical leaf. This lower rate of nuclear division is coupled with an apparently extra high stretching capacity in the walls, with the result that ingrowths of the wall towards the centre of the cell are formed. This is also illus-

trated by the very specialized type of mesophyll development found in *P. sylvestris* in which the whole of the assimilating tissue consists of 'arm' parenchyma developed from isodiametric cells. But in this leaf, the formation of 'arm' parenchyma can also be correlated with the fact that it is developed between two thick-walled and early differentiated layers, the endodermis and hypodermis. The formation of 'arm' palisade cells instead of the typical palisade suggests that possibly cellulose metabolism in these cells is initiated very early in development and is strongly maintained at the expense of protein metabolism, also that the spatial arrangements of the molecules of cellulose in the wall undergo a very rapid change. The meristematic and dividing stage in the cells of the spongy mesophyll is of short duration, giving fewer cells in this part of the leaf, but the longer period of vacuolation and extension produces cells of an irregular shape in between which the intercellular space system is developed.

Passing on now to a consideration of the range found in the development of the epidermal layers of the leaves described in this paper and also of its influence on the mature form of the leaf, the most striking example is illustrated by the development of the ericoid type of leaf. In these leaves, the upper epidermal cells vacuolate and enlarge very early in the development of the leaf, while the cells of the other tissues (except those of the main vascular bundle) remain small and meristematic, with the result that the leaf becomes rolled on either side of the main vascular bundle. In the epidermal cells, protein metabolism is of very short duration and the vacuolation and extending stages are initiated extraordinarily early in the developmental history of the leaf. In the ericoid leaves examined, the differentiation of the epidermis plays a very important part in determining the mature form of leaf, but in the other leaves described this does not seem to be the case, although various interesting differences in development were noted. In net-veined dorsiventral leaves, the upper epidermal cells are differentiated before those of the lower epidermis and they keep pace with the spread of the vascular supply and so form a somewhat rigid layer of cells filling up the interstices of the meshwork formed by the vascular bundles. They are differentiated before the layers of mesophyll cells below and consequently contribute something to the controlling forces under which these cells develop. Then in the parallel veined leaves of *A. aurantiaca* and *G. biloba*, it has already been noted that both layers of epidermal cells are differentiated at practically the same rate as the mesophyll cells. In *P. sylvestris*, the epidermis represents a highly differentiated protective layer with abnormally thick walls, on the outside of which is a heavy deposit of cuticle; the deposition of this cuticle results in the sinking of the stomata apparently into the layer below. Generally speaking, the duration of protein metabolism tends to be short in the cells of the epidermis of leaves, especially in those of the upper surface, and the phases

of extension are dominant in the development of this layer. In *Salix alba*, however, protein metabolism is maintained far longer than in the leaves of the other examples described, suggesting that the type of metabolism found in this layer must be fundamentally different to that characteristic of the majority of leaves.

The study of the development of foliage leaves involves the consideration of three points of view: (1) the development of the leaf as a complete unit, (2) the development of the different tissues composing the leaf, and finally, the development of the individual cells which make up the tissues. The last aspect of the study is the fundamental one, but it is by far the most difficult and involves a more delicate technique than we have at our command at present. The development of the different tissues can be studied more easily, by an extensive study of every type of leaf development, but that must of necessity be incomplete until we can correlate the cytological history of any tissue with the cycle of its physiological activities during development. Until this has been done we cannot really have any adequate idea of the interactions of developing tissues on each other, and it is a knowledge of this interaction which will eventually give us a correct picture of the leaf as a complete unit.

In conclusion, I should like to express my thanks to Professor Sir William Wright Smith for all the facilities provided in his department. My thanks are also due to the Royal Society for a grant defraying part of the expenses of the work.

#### SUMMARY.

1. An account is given of the development of the following foliage leaves: *Sambucus nigra*, *Pinus sylvestris*, *Alstroemeria aurantiaca*, *Ginkgo biloba*, *Erica baccans*, *Rosmarinus officinalis*, and *Salix alba* to illustrate some of the methods of tissue differentiation present in foliage leaves.

2. The type of mesophyll produced in the mature leaf is shown to be correlated with (a) the rate of nuclear division in the cells of the mesophyll layers, (b) the length of the duration of the three phases of cell differentiation in the cells of this tissue, (c) the rate and extent of the differentiation of the vascular supply, (d) the position of development of the mesophyll relative to the other leaf tissues.

3. Various types of epidermal development in foliage leaves are described. In ericoid leaves the rate of development of the upper epidermal cells was found to be an important feature.

4. The differentiation of the grooved leaf of *R. officinalis* was found to be correlated with the formation of an accessory parenchyma tissue.

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## EXPLANATION OF PLATES VIII AND IX

Illustrating Miss Tetley's paper on 'Tissue Differentiation in Some Foliage Leaves'. The drawings were done under a camera lucida, using a Zeiss lens D, na 0.65 ( $\times 620$ ).

Fig. 1. *Alstroemeria aurantiaca*. Part AB of leaf in text-fig. 6, 1.

Fig. 2. *A. aurantiaca*. Part of leaf at stage 2.

Fig. 3. *A. aurantiaca*. Part of mature leaf showing 'arm' palisade tissue.  
a.p. = 'arm' palisade cells.

Fig. 4. *Rosmarinus officinalis*. Part of leaf D, showing meristematic mesophyll cells and projecting multicellular hairs.

Fig. 5. *R. officinalis*. Part of leaf C, showing thick-walled cells developing in connexion with vascular bundle.

## PLATE IX

Fig. 6. *Salix alba*. Part of young leaf in bud.

Fig. 7. *S. alba*. Part of leaf showing early stage in vacuolation of upper and lower mesophyll cells.

Fig. 8. *S. alba*. Part of mature leaf, showing mesophyll tissue consisting of palisade shaped cells.







# Quantitative Observations on the Fixation and Transfer of Nitrogen in the Soya Bean, with Especial Reference to the Mechanism of Transfer of Fixed Nitrogen from *Bacillus* to Host.

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With one Graph in the Text.

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## I. INTRODUCTION.<sup>1</sup>

ALTHOUGH the literature dealing with bacterial symbiosis is very extensive, a number of problems require further investigation. One such problem is the method by which fixed nitrogen is transferred from bacterium to host. Taking as a working hypothesis the conventional view that the fixation occurs in the first instance within the bacteria themselves, then obviously some mechanism must exist to effect the transfer of some or all of this fixed nitrogen from the bacteria into the enveloping cytoplasm of the nodule cells, from whence the nitrogenous materials are transported by ordinary methods to various parts of the host plant. An increased understanding of the nature of this transferring mechanism would be a definite step forward in the elucidation of the real nature of the symbiosis. In recent papers, on the basis of his extensive investigations, Virtanen (12, 13) considers it to be probable that the host plant receives its nitrogen from the nodules in the form of amino acids, but no suggestion is made as to the form or mechanism of the previous transfer from bacterium into host cytoplasm.

Many earlier investigators, including Prazmowski, Frank, Nobbe and Hiltner, and Hartleb, reference to whose work may be obtained in a review

<sup>1</sup> A brief account (2) of the earlier of the experiments described in this paper appeared in 1933. [*Annals of Botany*, Vol. L. No. CXCIx. July, 1936.]



by Fred, Baldwin, and McCoy (5),<sup>↓</sup> considered the transfer to be effected as the result of a digestion of bacteria brought about by the enzymes of the host. This theory has on general grounds a certain attraction since bacterial and mycorrhizal symbiosis are thereby brought into line as regards the method by which food materials are supposed to be transferred from the lower to the higher symbiont.

Cytological studies of the nodule have yielded conflicting statements on the matter of digestion. The work of three more recent investigators will be considered. Thornton (10, 11) has examined the nodules of lucerne. He makes no mention of any disappearance of bacteria until the decay of the nodule sets in towards the close of the life-history of the host plant.<sup>✓</sup> Even then the degeneration of the host cell structures is described as preceding that of the bacteria, and as resulting from a parasitic attack by the latter, launched when a carbohydrate shortage arises at this period. After the destruction of the host nucleus, the bacteria lying in the cytoplasm break up into granules, and eventually disappear. A similar sequence is reported by Thornton for clover nodules.<sup>✓</sup> Milovidov (7) has investigated the cytology of the nodules of lupin, and he also makes no reference to a disappearance of bacteria until the final decay of the nodule, when the death of the bacteria and of the host cell are said to occur practically simultaneously.<sup>✓</sup>

Dangeard (4) has reported on the nodule cytology of a large number of leguminous plants. Unlike the two preceding authors, he states that in practically all the species studied by him, which included lucerne, the bacteria in relatively old nodules undergo a partial or complete disorganization, but are survived by the host nucleus. This is interpreted by him as a result of a digestion of the bacteria by proteolytic enzymes, secreted by the host cell. The nitrogenous products of digestion are, he suggests, utilized by the plant in the formation of its fruits and seeds.<sup>✓</sup>

The cytological study of the nodule must be regarded as incomplete. There may or may not be a digestion of bacteria in the cells of larger nodules. In any case, such digestive processes as described by Dangeard, only occurring in older nodules on plants in the reproductive phase, are inadequate to account entirely for the observed facts relating to the transfer of fixed nitrogen to the host plant. The latter process probably begins at a much earlier stage, since a nodulated plant displays signs of benefit soon after the first appearance of nodules.<sup>✓</sup> Dangeard himself realized this, and suggested that from the beginning of the symbiosis nitrogenous materials are obtained by the host plant as a result of the excretion of such materials by the bacteria into the surrounding host cytoplasm.<sup>✓</sup> The theory that transfer of fixed nitrogen occurs as the result of bacterial excretion did not originate with Dangeard, but appears to have been first advanced by Nobbe and Hiltner in 1893.<sup>1</sup>

✓Theories that transfer is effected by an autolysis of bacteria or by the action of a bacteriophage are referred to in the review by Fred, Baldwin, and McCoy (5). The lack of evidence for a disorganization of bacteria in younger nodules militates against these theories.✓

The quantitative study of the transfer of fixed nitrogen appeared to be a possible line of investigation. It is obviously desirable to know how soon transfer begins, whether it keeps place with fixation, or whether there is a lag between the two processes, due to the nitrogen being retained within the bacteria for a period. No previous investigator appears to have considered these matters. Data obtained by Whiting (19) give some information on the quantitative aspect of transfer, though not considered in that respect by him. Further reference to Whiting's data will be made in a later paper.

✓The quantitative study of fixation and transport has been complicated by the important results obtained in the last few years by Virtanen and his collaborators. Prior to the publication of their work there was no conclusive evidence for the escape of nitrogenous substances from the leguminous plant into the rooting medium, and the regular method employed for the measurement of fixation of nitrogen was to note the increase with time of the nitrogen content of host plant plus nodules, access to external sources of combined nitrogen being excluded. This procedure has been followed in the present experiments, which were partly completed before Virtanen's results came to the author's notice.✓ In view of the discovery by Virtanen and his associates (12, 13, 14, 16) that at any rate in certain legumes (pea, clover, and vetch) considerable quantities of the nitrogen fixed may leak out from the nodules into the rooting medium, it follows that the method described above measures what will be termed 'apparent fixation', i.e. 'real fixation' minus any fixed nitrogen that may escape into the rooting medium. This is not a serious detriment to the present investigation, as will be shown later.

Turning to transfer, there is no method of measuring directly the rate of movement of fixed nitrogen from the bacteria to the surrounding host cytoplasm, which, strictly speaking, constitutes the actual process of transfer.<sup>1</sup> One can, however, make observations on the subsequent movement of the nitrogen, which prior to Virtanen's work was visualized as being confined to a passage from the nodule to the root and thence to other parts of the plant. The rate of this transfer of fixed nitrogen from the nodules into the plant has been measured in the present work by noting, for plants having no access to external combined nitrogen, the increase in the nitrogen content of the *host plants apart from the nodules*. It is a reasonable assumption that the passage along the conducting channels from the

<sup>1</sup> The term 'transfer' is however used in this paper with reference also to the subsequent movement of the fixed nitrogen from the nodule into the rest of the plant or into the rooting medium.

nodules into the root of the fixed nitrogen liberated from the bacteria into the surrounding host cytoplasm occurs without appreciable delay.<sup>1</sup> If this is the case, then over a relatively long period practically all the nitrogen passing out of the bacteria will also pass out of the nodule within the period, so that a measurement of the exodus of fixed nitrogen from the nodules will also be a measure of the amount of nitrogen liberated from the bacteria. Virtanen's results suggest, however, that transference into the rest of the plant is not the only direction of export of fixed nitrogen from the nodules—some may leak out into the rooting medium. The term 'combined transfer' will be used with reference to the sum of transfer into the plant plus transfer into the rooting medium.

It is clear that the observations of Virtanen and his collaborators have rendered somewhat incomplete the data obtained in the experiments reported here. This is considered in the discussion, and it will be shown there that the value of the conclusions drawn from the present experiments has not been appreciably reduced by Virtanen's observations, which are largely complementary to those recorded here. Further experiments are, however, being planned in which measurement of the leakage from the nodules will also be made, but publication of the results already obtained seems desirable at this point.

## II. METHODS.

There was some variation of procedure in the different crops of plants. In this section only methods common to all the crops are considered, modifications made for individual crops being described in the next section.

The general procedure was as follows. Plants were cultivated under conditions designed to prevent any access to external sources of combined nitrogen, nodule development being ensured by suitable inoculation. Samples were taken from the plant population at frequent intervals, and for each sample the absolute total nitrogen content was determined of (*a*) the plants after removal of the nodules, and (*b*) the nodules. As explained above, the increase with time of the nitrogen content of plants less nodules is a measure of the rate of transfer of fixed nitrogen from the nodules to the host plant, while the increase of the combined nitrogen contents of plants and of nodules indicates the rate of fixation (apparent) of atmospheric nitrogen by the bacteria associated with the plants.

The Soya Bean ('Manchu' variety) has been employed in these experiments, the seeds being kindly supplied by Mr. W. J. Morse of the Bureau of Plant Industry, Washington, D.C. Seeds selected for weight were used.

The plants were grown in the glasshouse in washed nitrogen-free sand contained in pots, the nature of which varied for the different crops. In

<sup>1</sup> That this assumption is correct is proved by the results obtained (see later).

all cases distilled water was initially added to each pot of sand until a weight was attained which indicated that 12 per cent. of water was present, reckoned on the weight of the dry sand. A small excess of seeds was then laid on the sand, covered with moistened filter paper and left for three days, according to the procedure of Whiting (19). At the end of this period, when the radicles were just piercing the testas, any obviously diseased seeds were rejected and the number of seeds per pot adjusted to three more than the number of plants finally required. The seeds were then inoculated and sown. Inoculation was effected with a strain of *Bacillus radicicola* isolated from 'Manchu' Soya Bean, which was kindly supplied by Dr. I. L. Baldwin, University of Wisconsin. The seeds were dipped into a suspension of the bacteria immediately before sowing.

After two weeks seedlings were removed until the required number remained in each pot. The testas and any other parts falling from the plants were stored and added to the plant material during sampling. Every day or second day the moisture content of the sand was restored to 12 per cent. by the addition of distilled water or, from time to time, of a nitrogen-free culture solution (see below for formulae).

Samples were taken from the plant population at rather irregular intervals, the size of the sample varying in different experiments. The pots of plants comprising the samples were selected to be as representative as possible from the standpoint of size, and to this end, at each sampling all the pots of the experiment were graded into three groups on a basis of size, and a suitable number of pots from each group included in the sample.

It was next necessary to detach the nodules from the plants, and then to obtain with minimum loss (*a*) the combined dry matter of the plants less nodules from each pot, and (*b*) the dry matter of the nodules from each pot of plants. The contents of each pot were tipped out and the plants removed. The sand was subsequently dried and root fragments recovered from it by the use of a sieve. Sand adhering to the root systems was removed by washing, the wash water being sieved to recover root fragments. The nodules were then picked off and put to dry in the electric oven at 95° C. The plants themselves were cut up into small portions, and pulped with pestle and mortar, the material being subsequently transferred into an evaporating dish. The loss of material involved by the transference was shown by a test experiment to be considerably below 1 per cent. The plant tissues were then dried at 95° C. until the weight remained approximately constant.

The method used for estimating absolute nitrogen contents necessitated that after the final weight of the dry matter had been obtained, there should be no absorption of moisture prior to analysis. After the final weighing the plant dry matter was tipped into a dry mortar, pounded for one minute, and the pulverized material quickly placed in dry jars with

greased ground-glass stoppers. The jars were stored over calcium chloride in air-tight tins until the material was required for analysis. A check experiment showed that a weighed quantity of plant dry matter stored for two months in the above fashion gained in weight less than 0.1 per cent. over the period. The nodule dry matter was treated similarly.

The percentage total nitrogen contents of the plant and of the nodule dry matter from each pot were determined in triplicate by the salicylic acid Kjeldahl process as modified by Ranker (8, 9), and then by reference to the absolute weight of dry matter (determined before storage), the absolute nitrogen contents of plants and nodules were calculated. Preliminary determinations on Kahlbaum's asparagine gave a recovery of 99.1 per cent. of the nitrogen theoretically present.

To obviate any error arising from the adherence of dry matter to the inside of the neck of the Kjeldahl flask during combustion, the material was weighed out into small boats made from cigarette paper. The boats are for practical purposes free of nitrogen, and will slide down the neck into the body of the flask.

*Control plants.* With each crop of inoculated plants a group of pots of sterile non-nodulated plants were grown as controls, with the object of confirming that the inoculated plants had no access to combined nitrogen other than that derived from the bacteria. The seeds for these pots were each initially surface-sterilized by being dipped in absolute alcohol for ten seconds, and then being passed through a flame, a method employed by Whiting (19). The pots and sand were initially autoclaved and other suitable precautions taken to prevent infection by *B. radicola*.

### III. DATA AND FURTHER NOTES OF PROCEDURE.

#### 1932 plants.

Seeds weighing 0.17 to 0.21 gm. inclusive were used for the inoculated plants. Unglazed earthenware pots were employed, each containing  $5\frac{1}{2}$  kg. of silver sand, and while the majority of the pots were new and unused, a small proportion had been in use previously, and were slightly soiled. The culture solution employed was that of Bryan (3) except that calcium chloride was substituted for calcium nitrate. The pH of the solution was 7.2. Eight plants were grown in each pot, and three pots were taken at each sampling.

The data for this crop are presented in Table I. Nodules first appeared about three weeks after sowing. Since the nitrogen content of eight seeds was 90.3 mg., under the prevailing conditions of growth fixation does not appear to have commenced until after the fifth week. Examination of the figures for the nitrogen contents of the plants less nodules indicates that transfer of fixed nitrogen from the nodules to the rest of the plant com-

menced at the same time as fixation. It will be observed that at the first sampling date the nitrogen content of the plants less nodules (85.6 mg.) was slightly less than that of the original seeds (see above). The explanation may be that there is an export of plant nitrogen into the developing nodules.

The nitrogen contents of plants plus nodules and of plants alone are also presented graphically on p. 567. It is clear from the graph that throughout the development of the plant, a high proportion of the nitrogen fixed (and retained within the plant-nodule system) passes without delay from the bacteria and nodules into the rest of the plant. It follows of course that there is relatively little accumulation of nitrogen in the nodules (see Table I).

TABLE I.

*Data of 1932 Plants. The Figures in Columns 4-9 are Averages Per Pot, Based on the Examination of Three Pots, each Containing Eight Plants, the Plants (and also the Nodules) from each Pot being Bulkcd.*

Date of Sampling.	Age of plants (days from sowing).	Stage of development.	Number of nodules.	Dry weight of plants less nodules (gm.).	Dry weight of nodules (gm.).	N content of plants less nodules (mg.).	N content of nodules (mg.).	Combined N content of plants and nodules (mg.).
July 6	35	2nd trifoliate leaf opening.	126	3.18	0.095*	85.6	5.3*	90.9
" 14	43	2 trifoliate leaves expanded.	146	4.45	0.197*	111.4	11.4*	122.8
" 20	49	3 "	127	5.59	0.293	133.0	17.4	150.4
Aug. 3	63	4 "	170	9.01	0.618	214.8	34.0	248.8
" 10	70	5 "	227	10.76	0.861	261.7	47.6	309.3
" 24	84	6 "	287	14.64	1.306	398.1	65.9	464.0
Sept. 8	99	8 leaves. Abundant flowers.	362	21.03	1.976	560.7	95.3	656.0
" 17	108	Pods developing.	362	24.09	2.250	699.2	112.8	812.0
Oct. 4	125	" "	324	29.91	2.624	870.7	126.0	996.7
" 20	141	Pods mostly full. Leaves falling.	357	30.22	2.369	892.8	110.4	1003.2

\* The nodules from all three pots were bulked together at this sampling.

Also in the graph an indication is given of the extent of the variation in combined nitrogen content from pot to pot within the one sample. The variation of nitrogen content of plants less nodules was of course similar. Some such variation is inevitable except under ideal conditions. The fact that a smooth curve passing through all the mean points was obtained suggests that the samples taken were sufficiently large and representative.

The amounts of nitrogen fixed and transferred during successive experimental periods are presented in Table II. To facilitate comparison

between different periods values are also given on a daily basis. It is clear that transfer is throughout roughly proportional to 'apparent' fixation (for meaning, see p. 561), a point which is more clearly brought out by the ratios in the right-hand column of the Table. This relation between the two processes is considered in detail in the discussion. It may be noted that the maximum rates of fixation and transfer into the plant were reached at the flowering period. It cannot be definitely stated from these figures that 'real fixation' reaches its maximum at this period, but it is very probable that such is the case.

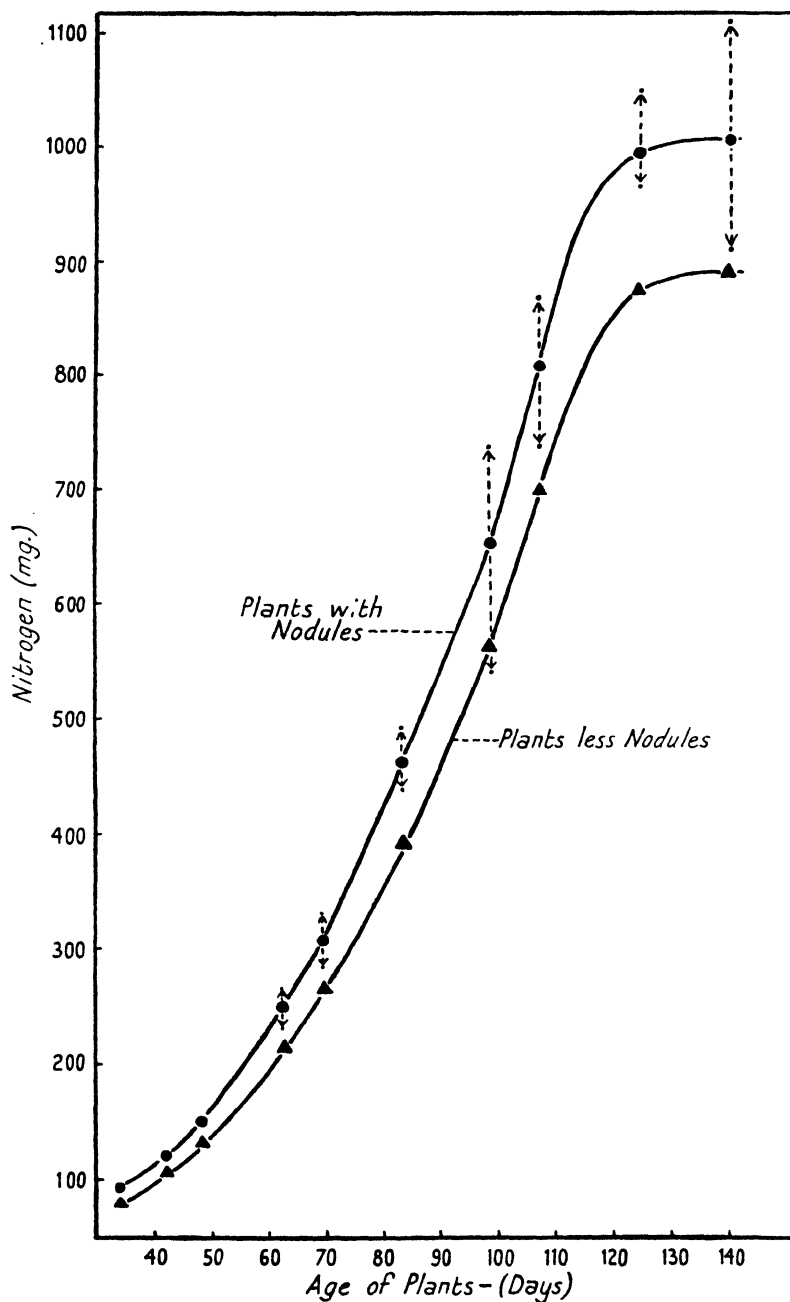
With the onset of the flowering stage, nodules undergoing decay were occasionally noticed on the plants. The proportion of decaying nodules increased somewhat towards the end of the experiment but was never very great. The decaying nodules were soft and burst under slight pressure, exuding a mass of disorganizing contents. Prior to the flowering stage there was no sign of any decay of nodules. (Similar observations were made on the plants of subsequent crops.)

*Controls.* Seeds weighing 0.16 to 0.18 gm. were used. In accordance with expectations the appearance of these plants soon indicated severe nitrogen starvation. The plants were grown for a considerable time before being removed for analysis, in order that any unsuspected access to combined nitrogen might be the more clearly revealed. The figures for these plants are presented in Table III. The combined nitrogen content of eight average seeds was 86.3 mg., and the figures for the controls are sufficiently near to this to prove that the plants had no external source of combined nitrogen. Since the inoculated plants, apart from the presence of *B. radicola*, were grown under the same conditions as the controls, it may be concluded that here also no nitrogen was available except for that derived from the bacteria.

#### *1934 plants.*

Seeds weighing 0.18 to 0.195 gm. inclusive were sown. Glazed earthenware pots were used for this crop, each holding  $3\frac{1}{2}$  kg. of a mixture of equal parts of silver sand and of a coarser sand. The pots and sand were autoclaved for three hours before use, and the seeds were surface-sterilized by the method used for the 1932 controls. The culture solution referred to by Warington (18) as 'Rothamsted nutrient solution pH 6.2' was used for this crop, with KCl substituted for  $\text{KNO}_3$ . Five plants were grown in each pot, and six pots comprised a sample.

The data for this crop are presented in Table IV. The growth and nitrogen fixation by this crop were inferior to those of the 1932 crop, due probably to the unsatisfactory aeration of the sand consequent on the use of glazed pots, and also to an unfavourable season climatically. The figures for nitrogen fixed and transferred during each of the experimental



Nitrogen content of eight plants with nodules and of eight plants less nodules at successive sampling dates (1932 plants). The arrows and smaller points show by how much the highest and lowest figures differed from the mean in the case of the plants with nodules.



periods are given in Table VI. There is the same correspondence between the two processes as was obtained in the 1932 experiment. The changes in nitrogen content during the last period in Table IV are so small as to be of very doubtful significance, and no figures for this period are given in Table VI.

TABLE II.

*Fixation of Nitrogen (Apparent) and Transfer of Fixed Nitrogen from Nodules into Plants during Successive Periods of Development (1932 Plants). The Data refer to Eight Plants.*

Period (days from sowing).	Fixation (apparent) of N * (mg.).	Amount of N trans- ferred from nodules to plants † (mg.).	Ratio of N trans- ferred to N fixed as % of N fixed.
35-43	31.9 (4.0) ‡	25.8 (3.2) ‡	80
43-49	27.6 (4.6)	21.6 (3.6)	78
49-63	98.4 (7.0)	81.8 (5.8)	83
63-70	60.5 (8.6)	46.9 (6.7)	78
70-84	154.7 (11.1)	136.4 (9.7)	87
84-99	192.0 (12.8)	162.6 (10.9)	85
99-108	156.0 (17.3)	138.5 (15.4)	89
108-25	184.7 (10.9)	171.5 (10.1)	92
125-41	6.5 (0.4)	22.1 (1.4)	350

\* Calculated by subtracting the nitrogen content of eight plants and their nodules at each sampling (see Table I) from the corresponding figure at the following sampling.

† Calculated by subtracting the nitrogen content of eight plants, less nodules, at each sampling (see Table I) from the corresponding figure at the following sampling.

‡ The figures in brackets represent fixation (second column) or transfer (third column) per day, obtained by dividing the total fixation or transfer for the period by the number of days in the period.

*Controls.* Nine pots each with five plants were grown, and the final combined nitrogen contents of the plants from the different pots were as follows: 60.8, 61.2, 63.0, 63.6, 59.2, 71.3, 58.7, 63.3, 56.8 mg. These are all sufficiently near to the combined nitrogen content of five average seeds such as were used for these controls (= 65.3 mg.) to prove that no nitrogen was available to the plants under the conditions employed. The first four pots of plants were two months old when taken out for analysis, and the remaining ones four months.

#### 1935 plants, I.

Seeds weighing 0.19 to 0.20 gm. inclusive were sown. The pots were of the type used in 1932, half of them being quite new, the remainder having been used for the 1932 crop only. Each pot contained 5½ kg. of the 1934 sand mixture. The pots and sand were autoclaved before use, but the seeds were not sterilized since experience showed that seeds with testas not perfectly intact were liable to be damaged. Eight plants were grown per pot, and each sample consisted of five pots. The nodules from the five pots were on this occasion bulked together in order to reduce labour. The culture solution was as in 1932.

The growth was better than in the previous year, and was similar to that of the 1932 crop, as the data of Table V show. The scale of this experiment allowed of sampling being continued until the stage of flower-bud formation only. The figure for the combined nitrogen contents of plants and nodules at the first sample is, it will be observed, lower than that of the original seeds. It is uncertain whether this is due to an actual

TABLE III.

*Details of the Non-nodulated Control Plants, 1932 Crop. The Eight Plants of each Pot were Bulkied for Sampling.*

Pot number.	Age of plants when analysed (weeks).	Dry weight of 8 plants (gm.).	N content of 8 plants (mg.).
1	12	4.22	83.5
2	"	4.17	81.4
3	"	4.20	79.3
4	"	5.40	86.8
5	16	6.02	91.1
6	"	5.07	75.4
7	"	5.76	87.0

loss of nitrogen during early development or to loss in the preparation of the dry matter.

Rates of fixation and of transfer from the nodules to the rest of the plant are presented in Table VI. Again there is a close correspondence between the two processes.

*Controls.* Eight control pots were grown. One was accidentally infected with *B. radicola* and consequently abandoned. The following are the nitrogen contents of the eight plants from each of the seven remaining pots: 87.2, 72.9, 80.0, 76.5, 78.0, 75.3, and 95.4 mg. The first two pots were removed for analysis after ten weeks growth, the others after sixteen weeks. The figures are all satisfactorily near to the nitrogen content of eight seeds, namely 88.4 mg., although in all the pots but the last there appears to have been some loss of nitrogen. A similar tendency can be detected in a number of the control pots of previous crops; the loss may be due to a decay of portions of the root system.

#### 1935 plants, II.

The procedure here was as for the preceding crop. The data are presented in Tables V and VI. Sampling was not begun until the plants were two months old, the plan being to use this crop for observations on later stages in development. Unfavourable weather slowed up development and little growth took place after the second sampling. Such small changes in nitrogen contents as are shown after this sample are of very doubtful significance.

TABLE IV.

*Data of the 1934 Crop. The Figures for each Date are Averages of Five Plants Per Pot based on the Examination of Six Pots, the Plants and the Nodules from each Pot being Bulkcd.*

Date of sampling.	Age of plants (days from sowing).	Stage of development.	Dry weight of plants less nodules (gm.).	Dry weight of nodules of plants (gm.).	N content of plants less nodules (mg.).	N content of nodules (mg.).	Combined N content of plants and nodules (mg.).
July 10	41	2 trifoliolate leaves.	1.74	0.05	60.3	2.9	63.2*
" 23	54	4 "	2.41	0.134	82.7	9.1	91.8
Aug. 6	68	5 "	4.60	0.326	131.9	17.2	149.1
" 20	82	7 "	5.46	0.432	165.0	22.0	187.0
Sept. 4	97	Flowers.	6.94	0.521	230.2	27.9	258.1
" 20	113	Flowers and young pods.	8.52	0.531	291.2	28.6	319.8
Oct. 10	133	Pods ripening.	9.23	0.481	295.1	24.3	319.4

\* The nitrogen content of five seeds was 60.2 mg.

## IV. DISCUSSION.

It must be stressed that the results obtained in the present experiments are based on the examination of relatively small samples taken from a plant population in which a certain variation was displayed. Some indication of the variation was given for the 1932 crop, and in the later crops the variation was of the same order, and larger samples were taken. It appears doubtful whether statistical treatment can satisfactorily be applied to the data in view of the relatively small scale of the experiments necessitated by considerations of space and labour. It is suggested, however, that the general consistency of the results of the different experiments is an indication that they are trustworthy.

In each experiment transfer of fixed nitrogen from the nodules, and therefore from the bacteria, into the plant began at approximately the same time as fixation. Further, the figures suggest that a close relation exists throughout between the rate of transfer of fixed nitrogen from the nodules into the rest of plant and the rate of fixation (apparent). The existence of this relation becomes still more obvious when the ratio of transfer to fixation is calculated for successive experimental periods (see Tables II and VI). For each crop the ratio remains relatively constant throughout the continuation of the experiment, although there is a tendency for the value to rise with the passage of time.<sup>1</sup> Taken collectively, the figures suggest that of the nitrogen fixed and retained within the plant-nodule system, a high proportion (over 75 per cent.) is regularly transferred from the nodules into the host plant.

These figures must now be considered in the light of the results obtained by Virtanen and his co-workers, to which reference has been made. If Virtanen's findings for other leguminous plants also hold for the Soya Bean, it appears that over and above the figures obtained in the present experiments, a further amount of nitrogen was fixed and subsequently escaped into the sand in the vicinity of the nodules. The position seems to be that of the nitrogenous substances liberated from the bacteria, only a proportion is collected by the vascular network enclosing the bacterial tissues and translocated into the plant; the remainder diffuses out through the cortical regions of the nodule and finally passes into the sand. Since transfer from the nodules into the plant commences with or very soon after fixation, as shown in the present investigation, we should expect the same to hold for transfer into the sand, an expectation which is realized in the observation made by Virtanen and his collaborators (16) that the leakage of nitrogenous substances from the nodules began almost

<sup>1</sup> It is considered that little importance can be attached to the very high figure for the final period in Table II, in view of the doubtful significance of such small changes in nitrogen contents as appear to have occurred in this period.

TABLE V.

*Data for 1935 Crops. The Figures for each Date are Averages based on the Examination of Five Pots, each of Eight Plants, the Plants from each Pot and the Nodules from all the Pots being Bulk together.*

Date of sampling.	Age of plants (days from sowing).	Stage of development.	Number of nodules on 8 plants.	Dry weight of 8 plants less nodules (gm.).	Dry weight of the nodules of 8 plants (gm.).	N content of 8 plants less nodules (mg.).	N content of 8 plants and their nodules (mg.).	Combined N content of 8 plants and their nodules (mg.).
1935, Crop I. (N content of 8 seeds, 86.4 mg.)								
July 9	29	2nd trifoliolate leaf opening.	44	2.13	0.036	79.0	2.1	81.1
" 19	39	3rd trifoliolate leaf opening.	80	3.58	0.162	96.1	9.4	105.5
Aug. 2	53	4 trifoliolate leaves expanded.	98	6.16	0.394	150.5	20.3	170.8
" 16	67	6 " "	113	9.60	0.795	233.9	34.3	268.2
" 28	79	7 leaves. Flower buds.	168	13.38	1.200	320.2	50.4	370.6
1935, Crop II. (N content of 8 seeds, 88.4 mg.)								
Aug. 26	56	5 trifoliolate leaves expanded.	87	6.46	0.544	201.4	27.0	228.4
Sept. 11	70	6 leaves. Flower buds.	147	11.12	1.184	297.2	50.0	347.2
Oct. 1	90	Flowers and small pods.	128	10.58	1.012	306.8	39.0	345.8

immediately after the first appearance of nodules, and thus soon after the initiation of fixation.

The amount of leakage may be extensive, for Virtanen (14, 16) found that in the Pea, during the earlier stages of development, as much as half of the nitrogen fixed may escape into the sand in this way. While we have at present no knowledge of the extent of this leakage in the case of the Soya Bean, it is quite clear that its occurrence means that the proportion of the fixed nitrogen that undergoes export from the nodules is

TABLE VI.

*Fixation (Apparent) and Transfer of Fixed Nitrogen from Nodules to Plants during Successive Periods in the Development of the 1934 and 1935 Crop, calculated from the Data given in Tables IV and V. For further explanation see Table II.*

Period (days from sowing).	Fixation (apparent) of N by bacteria of 5 or 8 plants (mg.).	Amount of fixed N transferred to 5 or 8 plants from their nodules (mg.).	Ratio of N transferred to N fixed as % N fixed.
1934 Crop (5 plants in each sample).			
41-54	28.6 (2.2)	22.4 (1.7)	78
54-68	57.3 (4.1)	49.2 (3.5)	85
68-82	37.9 (2.7)	33.1 (2.4)	89
82-97	71.1 (4.7)	65.2 (4.3)	92
97-113	61.7 (3.9)	61.0 (3.8)	97
1935 Crop I (8 plants in each sample).			
29-39	24.4 (2.4)	17.1 (1.7)	71
39-53	65.3 (4.7)	54.4 (3.9)	83
53-67	97.4 (7.0)	83.4 (6.0)	86
67-79	102.4 (8.5)	86.3 (7.2)	85
1935 Crop II.			
0-56	140.0 (2.5)	113.0 (2.0)	80
56-70	118.8 (8.5)	95.8 (6.8)	80

actually still higher than the ratios calculated above suggest, since the amount of nitrogen passing into the sand has to be added to both numerator and denominator of the existing ratios. Thus for any particular experimental period in the development of the plants:

Combined Transfer of Fixed Nitrogen to Plant and Sand

Fixation (real)

$$= \frac{\text{Transfer to Plant} + x}{\text{Fixation (apparent)} + x},$$

where  $x$  is the amount of nitrogen leaking out of the nodules into the sand during the period (see p. 561 for explanation of other terms). The result of this correction of the percentage ratios would obviously bring them still nearer to 100 per cent., so that the new values in all probability would lie between 80 and 100 per cent. The possibility, mentioned by Virtanen (16), must be borne in mind that a proportion of the nitrogenous substances leaking out of the nodules may subsequently be absorbed by the host plant

through its roots, more especially perhaps in later stages. It seems most probable that the gain of nitrogen from the sand in this way will be small compared with that taken directly from the nodules, but the occurrence of any such re-absorption will make the figures obtained in the present work approximate more closely to the correct figures for real fixation and combined transfer.

After making full allowance for the possible effect of these adjustments on the figures obtained, one is still justified in concluding that *from the commencement of fixation and onwards, a high proportion of the nitrogen fixed by the bacteria, probably 80-90 per cent., is regularly liberated into the host cytoplasm and is then transferred to other parts of the plant or into the rooting medium. The rate of transfer of fixed nitrogen from the nodules is roughly proportional to, and is doubtless governed by, the current rate of fixation.* The fixed nitrogen is not 'locked up' inside the bacteria or nodule cells<sup>1</sup> for any appreciable period before being subject to transfer. With such storage, transfer relative to fixation would be low during the earlier stages and higher later, when it might then even exceed the current rate of fixation. At the same time higher nitrogen contents for the nodules would be obtained in the earlier stages when accumulation was in progress. It may be noted that Virtanen (14) finds for the pea that the leakage of nitrogen into the sand is more extensive in pre-flowering stages than in later. If the same applies to the Soya bean, then of the total fixed nitrogen exported from the nodule, relatively less finds its way into the host plant in the earlier stages than in the later.

The conclusions drawn above will now be considered in relation to the mechanism of transfer of fixed nitrogen from the bacteria to the surrounding host cytoplasm. The results obtained confirm the point made in the introduction that such digestion of bacteria as has been cytologically observed is inadequate as a complete explanation of transfer. The possibility must, however, be mentioned that extensive digestion does occur within the bacterial tissue from the beginning of the symbiosis, even in the *relatively small and still-growing* nodules which characterize the plant during the earlier stages of its development, and that this digestion has been overlooked by cytological investigators. Each bacterium might be considered to remain for a time immune to any digestive propensities of the host cell, but subsequently to lose that immunity and to undergo digestion, with the result that most of the nitrogen stored up within the bacterium would be rendered available to the host. On this basis the observations made in the present work can be explained, provided that every bacterium passes through this cycle in much the same time, and that the cycle is completed fairly rapidly, say in several days. Otherwise there would be a lag between fixation and transfer, leading to low relative values of

<sup>1</sup> The assumption made on p. 562 is thus justified.

transfer during earlier phases. By parallel arguments an attempt could be made to explain the observations of this paper on the basis of the theories of transfer by autolysis or bacteriophage action. The drawback to any of these explanations is the absence of cytological evidence for a digestion or disappearance of bacteria in the earlier stages of development.

It appears to the author that a more satisfactory explanation of the observations is furnished by the idea of a passive excretion of nitrogenous materials by the bacteria. It is likely that with such a mechanism the rate of transfer will be controlled by the rate at which products of fixation are formed within the bacteria. There is the difficulty that the bacteria appear to lose as much as 90 per cent. of the nitrogen fixed. This difficulty diminishes when it is recalled that the fixation of nitrogen by *B. radiculicola* may be an exothermic process, to be regarded primarily as a form of respiration, comparable to the respiration of the sulphur and nitrifying bacteria, rather than as a special method of obtaining nitrogen for protein synthesis. Efforts to estimate the amount of host carbohydrate consumed by the bacteria have yielded conflicting results (5). In a recent paper, Virtanen (12) considers that the fixation is a reduction, the necessary hydrogen being obtained from sugars through a butyric fermentation which the bacteria have been shown (17) to promote. If the fixation consists of the direct reduction of nitrogen to ammonia, it would seem that energy will be released, since this reaction is exothermic.<sup>1</sup> Under these circumstances the fixation is perhaps rightly regarded as a form of respiration, in which case, as pointed out by Göbel (6), it might be expected that there would be a constant diffusion of nitrogenous end-products out of the bacteria into the surrounding host cytoplasm. It is unfortunate that the excretion of nitrogenous materials by the bacteria cannot be shown directly, for according to the latest investigations (5) the organism does not fix nitrogen under such conditions of pure culture as have yet been devised.

No evidence has been obtained in favour of the suggestion of Dangeard (4) that transfer by excretion is supplemented by digestion in the later stages. There is nothing in the results to indicate that a secondary mechanism of any importance comes into operation at an intermediate stage. The relatively low nitrogen content of the nodules throughout precludes any such process.

While the data of the present experiments bear chiefly on the problem of transfer, they are also of interest in other respects. An approximate index of the efficiency of the nodule tissues in the fixation of nitrogen over successive periods has been calculated from the data as follows:

$$\text{Efficiency in fixation} = \frac{F}{\frac{W_1 + W_2}{2}},$$

<sup>1</sup> Virtanen (15) does, however, speak of energy being required for fixation.



where  $F$  is the apparent fixation in mg. per day over a given experimental period by the bacteria associated with a pot of plants,  $W_1$  and  $W_2$  being the dry weight in gm. of the nodules on the plants at the beginning and the end of the period. The value thus obtained of course represents the daily fixation (apparent) of nitrogen by the amount of nodule tissue productive of 1 gm. of dry matter. If, as Virtanen's work indicates, the nitrogen

TABLE VII.

*Efficiency of Fixation by the Nodules during Successive Periods of Development of the 1932 and 1935 (I) Plants.*

Period (days from sowing).	Mean dry weight of nodules of 8 plants during period (gm.).	Daily fixation (apparent) of N by bacteria of 8 plants during period (mg.). (Figures from Tables II and VI.)	Efficiency (mg. N fixed per day by 1 gm. nodule dry matter).
1932 plants.			
35-43	0.146	4.0	27.6
43-49	0.245	4.6	18.4
49-63	0.456	7.0	15.4
63-70	0.740	8.6	12.0
70-84	1.084	11.1	10.3
84-99	1.641	12.8	7.8
99-108	2.113	17.3	8.1
108-25	2.437	10.9	4.5
125-41	2.496	0.4	0.2
1935 plants I.			
29-39	0.099	2.4	24.2
39-53	0.278	4.7	16.9
53-67	0.595	7.0	11.8
67-79	0.997	8.5	8.5

determined in the present experiments does not represent the total fixation, then the figures calculated as above underestimate the efficiency of the nodule tissues, and especially in the earlier stages, since it is then that the leakage is greatest.

The values obtained for this efficiency in fixation are presented in Table VII for two crops. Very similar figures were found for the other crops. It will be observed that with one exception the value falls throughout. The consideration mentioned in the previous paragraph suggests that actually the efficiency falls still more steeply. The only explanation of such a marked decrease in the value of the index is that there is a falling off in the average activity of the bacteria with the passage of time, even during the period when the rate of fixation by the whole population of bacteria associated with the plant is on the upgrate.

This reduction in average activity in fixation might be ascribed to an increasing competition for available carbohydrate as the bacterial population expands, the carbohydrate being required for processes associated with fixation. Another possibility is that after an initial period of active

fixation, the individual bacterium may pass spontaneously into a state in which fixation is reduced or ceases, the function of fixation constantly passing on to new generations of bacteria. This would lead to the presence within the nodule of an ever-increasing number of inactive bacteria. The loss of activity might coincide with the assumption of the bacteroid form, as suggested by Bazarewski (1).

It remains to be seen to what extent the conclusions drawn from this work may be extended to leguminous plants in general. Preliminary experiments with the garden pea (*Pisum sativum* L.) have yielded results similar to those for the Soya bean.

#### V. SUMMARY.

1. An attempt has been made to determine in nodulated Soya bean plants the rate of transfer of fixed nitrogen from the bacteria to the host over successive periods in the life cycle of the latter, and to observe the relation of this rate of transfer to the rate of fixation of nitrogen.

2. Taken in conjunction with Virtanen's recent results, the present investigation shows that from the commencement of fixation and onwards, of the nitrogen fixed by the bacteria a very high proportion, probably in the region of 80-90 per cent., is regularly liberated without appreciable delay into the host cytoplasm and undergoes transference to other parts of the plant or the rooting medium. The rate of transfer is thus proportional to, and probably governed by, the prevailing rate of fixation. There is no retention or storage to any considerable extent of the fixed nitrogen within the bacteria or the nodules.

3. At present these conclusions seem to accord best with the theory of transfer by passive excretion of nitrogenous substances by the bacteria, especially if fixation is taken to be a form of bacterial respiration rather than part of the process of bacterial protein synthesis. If the fixation is a respiratory process one would expect a large proportion of the products of fixation to pass out of the bacterial cell.

4. During the development of the plant there is a constant falling-off in the average activity in fixation of the bacteria within the nodules. This may be due to a decreasing supply of carbohydrates to the individual bacterium, or alternatively to the presence within the nodules of a constantly increasing number of inactive bacteria.

In addition to acknowledgments already made, the author is indebted to Professor V. H. Blackman, Dr. S. Williams, and Dr. H. G. Thornton for suggestions made during the course of the investigation. The greater part of this work was carried out during the tenure by the author of a

Carnegie Teaching Fellowship. Thanks are also due to the Carnegie Trustees for a grant towards the cost of apparatus and of publication. Mr. A. R. Gemmell gave valuable assistance with routine work of the 1934 experiment.

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# Physiological Studies in Plant Nutrition.

## V. Carbohydrate Metabolism in Relation to Nutrient Deficiency and to Age in Leaves of Barley.

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With ten Figures in the Text.

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## INTRODUCTION.

THE results of previous work on the relation of growth and respiration to nutrient deficiency in the barley plant directed attention to the need for information on the nitrogen and carbohydrate metabolism of leaves from such plants grown under standard conditions of manurial deficiency. It appeared necessary to ascertain the actual amounts of sugars and various nitrogen fractions present in the successive leaves of the main axis, i.e. in leaves similar to those used in the respiration experiments. The results of the nitrogen analyses are discussed elsewhere by Richards and Templeman (37); in this paper the data for carbohydrates are dealt with. Previous work in this field shows that no systematic investigation of the relation of carbohydrate metabolism to manurial deficiency has yet been undertaken, and as the findings of various workers relate to different plants and are not in agreement, it appeared necessary to carry out a comprehensive study *ab initio* of the leaves of barley grown under the same standard conditions as were used in the previous investigations in this institute.

## HISTORICAL.

In considering earlier work on the effects of manurial deficiency, the deficiencies in nitrogen, phosphorus, and potassium are dealt with separately.

In the tomato increases in reducing sugars, sucrose, and polysaccharides due to nitrogen deficiency are reported by Kraus and Kraybill (18), Kraybill (19), and Kraybill and Smith (20). In the sugar-cane addition of ammonium sulphate leads to a decrease in sucrose content, according to Kerr (17). Orcutt and Wilson (32), working with soybeans, conclude that a medium nitrogen supply results in an increase of soluble sugars, while a high or a low nitrogen level reduces the soluble sugar content. Low nitrogen supply results in lower glucose content, but the ratio of sucrose to reducing sugars is higher and the ratio of starch to hexose higher still.

Phosphorus deficiency has been investigated fairly extensively in the tomato plant. Kraybill and Smith (20), Kraybill (19), and MacGillivray (23) all find high reducing sugar, high sucrose, and high total carbohydrate content. Eckerson (7) finds a high starch and sugar content in leaves and stems in the early stages, but later a fall in starch content with increase in soluble sugar. Kerr (17) records a slight reduction in sucrose in the sugar-cane as a result of application of superphosphate.

The effects of potassium deficiency appear to have been much more fully investigated. As early as 1870 Nobbe (30) drew attention to the importance of potassium for carbohydrate formation, and this is well shown in the yields of mangolds from the Barnfield experiment at Rothamsted (40).

More recently Stoklasa (42) has shown that potash supply increases total carbohydrates. Phillips and Smith (33), working with tomato and radish, showed that a reduction of potassium supply to one-tenth leads to marked reduction in carbohydrates, and that practically no sugar is present in complete absence of potassium. Decreases in soluble sugars and starch in consequence of a low potassium supply are reported for sugar-beet by Leibrandt (22) and Rosenblüh (39), for potatoes by Niklas and Schropp (29) and Lebedyanzev (21). Janssen and Bartholomew (15) report an increase in sugars, starch, and dextrin in soybeans, cowpeas, sudan grass, clover, and corn, on raising the level of potassium. They note however that this relationship may be reversed in young plants. In a later publication (16) the same investigators note a higher reducing sugar and total sugar content in the cowpeas and sugar-beet under potassium deficiency.

A number of investigators have found quite the reverse effect of potassium deficiency: namely, an increase in sugar and starch under conditions of a low potassium supply. Thus in the tomato Phillips, Smith, and Dearborn (34), in the potato Burt (2), in the sugar-cane Hartt (14), in the pumpkin Burrell (3), all find starch accumulation under potassium starvation. Recently also potassium deficiency has been shown by White (48) to lead to starch accumulation in *Lemna*. The contradictory findings of the investigators mentioned above show that the question of the starvation effect of potassium is complicated by the effect of the other ions present, mainly calcium and sodium. The matter is discussed by Richards and Templeman (37) in relation to nitrogen metabolism where a similar confusion is found in the literature.

Gassner and Goeze (9) have shown that a low potassium supply reduces the assimilation rate in wheat seedlings, and confirm a similar finding for barley by Gregory and Richards (12). Finally, Maskell (24) has shown that potassium sulphate increases translocation rate in the potato.

#### EXPERIMENTAL PROCEDURE.

The plant used in this investigation was a pure line Barley, var. 'Plumage Archer'. The plants were grown in the open at Rothamsted in sand culture, the method used being similar in all respects to that described for previous experiments (11, 12), and given in some detail by Richards and Templeman (37). Approximately 200 pot cultures were used to obtain material for analysis.

Four series of plants were used, the standard manurial treatment (for convenience referred to subsequently as FM), and nitrogen, phosphorus, and potassium deficient series (referred to as -N, -P, -K), each receiving respectively one-tenth the amount of the particular nutrient element in deficit as was given to the fully manured series.

*Manurial scheme.*

The manurial scheme is presented below, the figures given being the weight of salts added per pot. In the deficient series the sodium content was adjusted to that of the fully manured series by the addition of sodium sulphate.

TABLE I.

*Manuring Scheme: Amounts in Gm. per Pot.*

	NaNO <sub>3</sub> .	Na <sub>2</sub> HPO <sub>4</sub> .12H <sub>2</sub> O.	K <sub>2</sub> SO <sub>4</sub> .	Na <sub>2</sub> SO <sub>4</sub> .10H <sub>2</sub> O.
Fully manured	9.1	2.524	1.85	none
Nitrogen deficient	0.91	2.524	1.85	15.52
Phosphorus deficient	9.1	0.252	1.85	2.044
Potassium deficient	9.1	2.524	0.185	none

In addition every pot received 1.25 gr. MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.37 gr. CaCl<sub>2</sub>.6H<sub>2</sub>O, trace of iron (FeCl<sub>3</sub>), and manganese sulphate.

Two experiments were carried out, in 1931 and 1932, and the sowing dates in these two years were May 3rd and May 6th respectively. In 1931 the whole bulk of manure was added to the pots within a few days of germination, whereas in 1932 the manures were applied in three fortnightly doses, the total, however, being the same in both years. The plants were watered with tap water, the aim being to maintain the water content of the sand as nearly as possible similar in all the series irrespective of the size of the plants.

As the symptoms induced by manurial deficiency are given in considerable detail by Richards and Templeman (37), it is not necessary to describe them fully here. The nitrogen-deficient plants were much reduced in size, the tillering was markedly less, and the leaves from the fourth onward were smaller in size and pale green in colour. Phosphorus-deficient plants showed in the later leaves a typical red coloration, the last leaves, however, becoming more normal in appearance. Tillering was much reduced and earing considerably delayed. The potassium-starved plants showed from the fourth onward the light green succulent leaves typical of potassium starvation under conditions of low calcium and high sodium manuring. These plants tillered freely and showed the well-known phenomena of a rapid death of the individual leaves and failure in stem elongation and ear emergence.

The behaviour of the plants in the two years was somewhat different; those of 1932 showing the starvation symptoms earlier, and stem-elongation in this year was delayed in all sets.

*Method of sampling of the leaves.*

The aim of these experiments was to investigate the sugar content of individual leaves of known morphological status and in comparable stages of development. The leaves of the main stem were alone used. It was

therefore necessary to be able with certainty to identify the main shoot and the serial number of the leaves. For this purpose the first and then every odd-numbered leaf on the main stem was marked on the auricle with waterproof ink. The leaves were removed for analysis at the time when they had reached full development, and on each such occasion as many of the previous leaves as were still living were also removed, so that in this way not only were the leaves analysed at the time of full emergence, but in subsequent stages of senescence also. Plants were only used for one sampling, after which they were discarded; this necessitated a large number of pot cultures, in all—as noted above—approximately 200.

Two practical difficulties were encountered in the sampling: firstly, the weather conditions were liable to vary from day to day so that it was imperative for the purpose of comparison between the treatments to sample all series on the same day; secondly, the rate of production of leaves differed in the series. It was always found that fully-manured and potassium-deficient series had exactly the same leaf-production rates; whereas in the nitrogen-deficient series, and even more so in the phosphorus-deficient series leaf production was slower. For any one day therefore, and for any particular leaf, the fully-manured and potassium-deficient series were more advanced than the nitrogen-deficient and phosphorus-deficient series; a compromise therefore had to be made in selecting sampling days. This objection is not so serious, as all the leaves were sampled at varying stages of senescence, and therefore by comparing within treatments the results of all analyses made on any one leaf the general effects of manuring become apparent.

In spite of the large differences in growth and the varying leaf-production rates in the different series, the total number of leaves produced on the main stem remained remarkably constant in all series. Applying the manures early in one dose resulted in the production of usually ten leaves, whereas with later additions of manures eleven leaves were produced.

The method of sampling adopted was the following: the leaves in the desired state were removed from three separate plants taken at random from different pots, severed at the base and placed in corked tubes lined with moist filter paper. They were taken to the field laboratory and their fresh weights immediately determined by weighing on a torsion balance, reading to the nearest milligram, or with larger leaves on a chemical balance to the nearest centigram. Each individual determination is therefore based on the average of two or three leaves taken at random. In general, three such samples were taken for each specified leaf, and as each of these samples was purely random it was possible to determine the random sampling error. After determining the fresh weight, the leaves were cut into pieces and plunged into boiling 95 per cent. alcohol, in which they remained for fifteen minutes to ensure inactivation of the enzymes. The



tubes were then stoppered and kept for analysis. The dates on which the various samples were taken are as follows :

1931. (1) May 29, (2) June 8, (3) June 12, (4) June 22, (5) June 29, (6) July 6, (7) July 17, (8) July 22.

1932. (1) June 3, (2) June 11, (3) June 15, (4) June 18, (5) June 20, (6) July 1, (7) July 8, (8) July 21.

In the diagrammatic representations of the analytical results the date of sampling is either stated or the sample numbers are given so that the dates of sampling may be identified.

#### *Extraction of sugars.*

Before analysis the sugars were completely extracted from the leaves by a further boiling in alcohol for eight hours. The tubes were immersed in a water-bath maintained at 85° to 90° C., and loss of alcohol prevented by the use of a reflux condenser. To prevent 'bumping' it was found advantageous to place in each tube one or two short lengths of fine glass capillary closed at one end.

After extraction the capillaries were broken with a glass rod and the contents of the tubes transferred to a distillation apparatus, together with washings of the leaf material. The alcohol was then evaporated off under reduced pressure at 30° C., and to avoid evaporation to dryness 5 c.c. of water were added to each tube before distillation. Removal of alcohol was taken to be complete when boiling ceased and no detectable smell of alcohol vapour remained. The contents of the tubes were diluted and transferred to a graduated flask. The extract was then clarified by the addition of basic lead acetate (specific gravity 1.25) at the rate of 0.1 c.c. per 0.2 gm. fresh weight of leaf. The excess lead was removed with saturated sodium phosphate (three times the volume of lead acetate used). The contents of the flask were immediately made up to the mark—the volume occupied by the precipitate was found to be negligible—the precipitate filtered off, and the solution kept in stoppered glass bottles with addition of toluene.

#### *Method of sugar determination.*

The sugars were estimated by the Hagedorn and Jensen (13) method on the original micro-scale. All determinations were in duplicate. A blank consisting of the same volume of the test solution and 2 c.c. ferricyanide was done for every set analysed; these were kept in ice from the time the ferricyanide was added until they were titrated. For the determination of total sugars acid inversion was used throughout.

### EXPERIMENTAL RESULTS.

The data of the sugar analyses are presented in Tables II and III, and are shown graphically in Figs. 1 to 8. Of the data here presented

there are two types: (1) the sugar content of the leaves at maturity, (2) the sugar content of the leaves at varying stages of senescence.

In previous papers dealing with the physiological behaviour of leaves of the barley plant (12, 36), it has been stressed that these were analysed

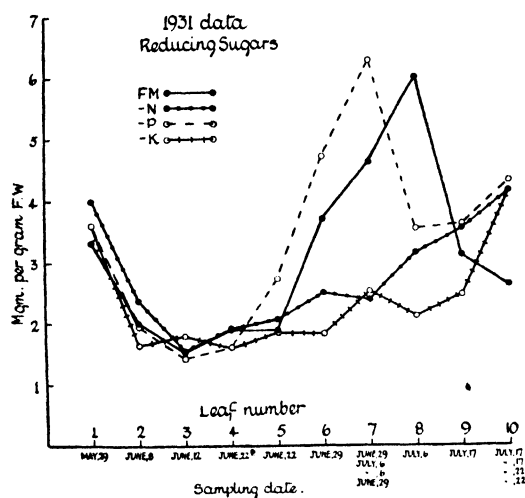


FIG. 1. Reducing sugar content (mg. hexose per gramme fresh wt.) at time of full expansion of successive leaves on the main axis in the four manurial series. Sampling dates as indicated. Manures applied in one dose at germination.

at stages of development as nearly as possible comparable, i.e. successive leaves were analysed at stages as near as possible to that of first reaching full development. This apparently has not been everywhere understood, since the work of Gregory and Richards (12) has been adversely criticized by Gassner and Goeze (9) on the grounds that analyses were made of leaves at different stages of senescence. Apparently these authors, whose criticisms have been quoted in subsequent papers, were under the impression that all the leaves on the plant were removed at one time. The inadequacy of this very method has again been stressed in a recent paper by Richards (38). In this work, unless otherwise stated, all analyses were performed on leaves at comparable stages of development.

In the Tables (II and III) and Figs. (1 to 8) the sugar contents are presented on the basis of mg. hexose per gm. of fresh weight.

The data for the two years in which the experiments were performed are presented separately. Reference may once more be made to the fact that the method of manuring was different in the two years. In 1931 the manures were applied soon after germination; in 1932 an equal total amount of manure was applied in three fortnightly doses during a period roughly corresponding to that of the emergence of the first six leaves.

TABLE II. 1931 Data.

*Sugar Content of Leaves at Emergence and at Varying Stages of Senescence, expressed as Mg. Hexose Per Gm. Fresh Weight, and the Ratio of Sucrose to Reducing Sugars, in the Four Treatments Studied. Each Entry is the Mean of Two or More Replicate Samples.*

Leaf No.	Sample No.	Fully matured.			Nitrogen deficient.			Total sugars.	Sucrose.	
		Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.	Reducing sugars.		Reducing sugars.	Sucrose.
1	1	3.32	21.01	24.33	4.00	18.47	4.00	22.47	4.62	4.62
	2	3.12	21.85	24.97	4.17	27.76	4.17	31.93	6.66	6.66
	3	2.29	16.16	18.45	2.64	20.15	2.64	22.79	7.63	7.63
2	2	2.00	8.24	10.24	2.36	19.67	2.36	22.03	8.33	8.33
	3	2.04	14.62	16.66	1.94	16.81	1.94	18.75	8.66	8.66
3	3	1.56	21.76	23.32	1.54	23.24	1.54	24.78	15.09	15.09
	4	2.11	25.33	27.44	2.09	29.41	2.09	31.50	14.07	14.07
4	4	1.88	29.54	31.42	1.91	36.78	1.91	38.68	19.26	19.26
	4	1.87	17.51	19.38	2.05	36.98	2.05	39.03	18.04	18.04
5	5	3.53	25.31	28.84	2.54	43.91	2.54	46.45	17.29	17.29
	6	2.34	14.17	16.51	—	—	—	—	—	—
6	5	3.70	32.65	36.35	2.48	61.56	2.48	64.04	24.82	24.82
	6	3.05	15.69	18.75	2.95	48.43	2.95	51.38	16.42	16.42
7	5	4.62	36.92	41.54	—	—	—	—	—	—
	6	3.31	20.76	24.07	2.37	43.81	2.37	46.18	18.49	18.49
	7	2.21	7.01	10.32	3.47	42.47	3.47	45.94	12.24	12.24
8	7	7.55	19.56	27.11	4.03	49.14	4.03	53.17	12.19	12.19
	6	5.99	29.49	35.48	3.14	37.86	3.14	41.00	12.06	12.06
	7	2.62	16.62	19.24	3.19	50.04	3.19	53.23	15.69	15.69
9	8	5.18	20.16	25.34	3.30	49.18	3.30	52.48	14.90	14.90
	7	3.10	20.77	23.87	3.53	43.68	3.53	47.21	12.37	12.37
10	8	6.19	28.59	34.78	3.27	43.68	3.27	46.95	13.36	13.36
	7	2.61	19.90	22.51	4.14	19.36	4.14	23.50	4.68	4.68
	8	7.86	26.58	34.44	6.07	33.67	6.07	39.74	5.55	5.55

TABLE II (*continued*).

Leaf No.	Sample No.	Phosphorus deficient.			Potassium deficient.			Sucrose.	
		Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.
1	1	3.60	18.29	21.89	3.58	19.43	23.01	5.43	5.43
	2	3.18	24.79	27.97	3.34	17.08	20.42	5.11	5.11
	3	1.99	24.78	26.77	1.84	15.12	16.96	8.22	8.22
2	2	1.93	17.86	19.79	1.63	8.00	9.63	4.91	4.91
	3	1.55	20.81	22.36	1.67	14.47	16.14	8.66	8.66
3	3	1.46	22.07	23.53	1.78	18.43	20.21	10.35	10.35
	4	1.95	20.78	22.73	2.06	6.67	8.73	3.24	3.24
4	4	1.61	20.93	22.54	1.59	12.02	13.61	7.56	7.56
	5	2.72	24.70	27.42	1.84	15.49	17.33	8.42	8.42
	6	5.22	17.28	22.50	2.07	5.17	7.24	2.50	2.50
6	5	4.71	15.92	20.63	1.82	5.71	7.53	3.14	3.14
	6	7.00	16.62	23.62	2.23	2.69	4.92	1.21	1.21
7	5	—	—	—	2.51	13.19	15.70	5.25	5.25
	6	6.26	18.81	25.07	1.59	4.42	6.01	2.78	2.78
	7	—	—	—	1.85	2.25	4.10	1.21	1.21
8	6	3.56	19.84	23.40	2.10	8.62	10.72	4.10	4.10
	7	4.56	18.69	23.25	1.85	6.97	8.82	3.77	3.77
	8	7.25	28.35	35.60	2.08	2.81	4.89	1.35	1.35
9	7	3.60	18.51	22.11	2.45	11.65	14.10	4.76	4.76
	8	4.52	24.32	28.24	2.97	11.00	13.97	3.70	3.70
10	7	—	—	—	—	—	—	—	—
	8	4.31	19.85	24.16	4.15	22.28	26.43	5.37	5.37

*Reducing sugars. 1931 data.*

The reducing sugar content of the successive leaves at the times of reaching full development is shown graphically in Fig. 1. The values here plotted correspond with the first entries for successive leaves in Table II. The actual sampling dates are entered in the figure below the leaf numbers.

For convenience of reference the fully-manured, nitrogen-deficient, phosphorus-deficient, and potassium-deficient series are referred to subsequently as FM, —N, —P, and —K respectively.

Up to the time of development of the fourth leaf there is little difference to be seen in the manurial treatments. All the curves show a high value for the first leaf which rapidly falls to the third leaf. From the fourth leaf onwards manurial differences become very apparent. The FM plants show no change until the fifth leaf, after which the hexose content rapidly rises, reaching a well-marked maximum at the eighth leaf, with a subsequent rapid fall to the tenth leaf in which the sugar content is rather less than the original value for the first leaf.

The —N series shows after the fourth leaf a very slow uniform rise in sugar content always below that of the FM until the ninth leaf, and finally reach in the tenth leaf a value identical with the original value.

Plants of the —P series show from the fourth leaf onwards a rapid rise reaching a maximum at the seventh leaf followed by a rapid fall in the eighth leaf, and finally a rise to the original value in the tenth leaf.

Plants of the —K series resemble those of the —N rather closely in that after the fourth leaf there is a slow rise which continues to the ninth leaf, and only at the last leaf is there a rapid rise to the original value. After the fourth leaf the values of the —K series are always below those of any of the other series with the single exception of the seventh leaf which is slightly higher than the corresponding leaf in the —N series.

In general therefore the —P more closely resembles the control plants and differs markedly from the —N and —K series.

*Total sugars. 1931 data.*

The fluctuations in the total sugar content in the FM series are not so great as in those for free hexose. In the first leaf the total sugar content in all series is the same, but in the FM the value falls in the second leaf, then rises and reaches a first maximum in the fourth leaf. A fall occurs in the fifth leaf when a cycle of changes begins, reaching a second maximum in the seventh leaf and a subsequent return to the initial value in the tenth leaf.

In contrast with the reducing sugars the —N series always shows values higher than those of the control, with the exception of the first and last values which are the same. The total sugar content in this series shows little change up to the third leaf, but subsequently a very rapid

increase occurs, reaching a maximum in the sixth leaf with a somewhat irregular fall to the initial value in the tenth leaf.

The —P series show a remarkably constant total sugar content throughout the whole succession of leaves, only minor irregularities occurring.

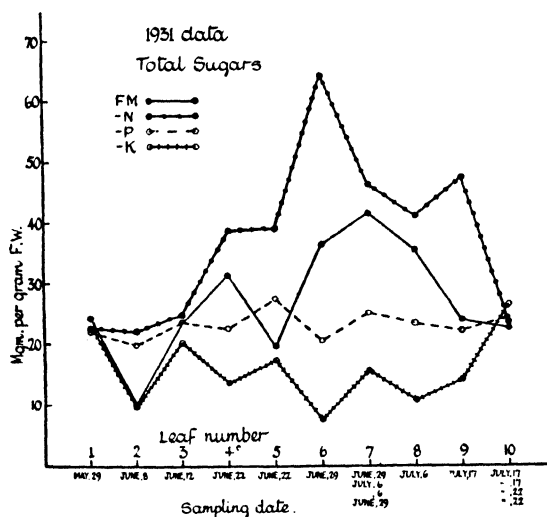


FIG. 2. Total sugar content (mg. hexose per gramme fresh wt.) at time of full expansion of successive leaves on the main axis in the four manurial series. Sampling dates as indicated. Manures applied in one dose at germination.

The —K series follows the control in showing a fall at the second leaf and a subsequent rise, but this is not maintained and the curve shows an irregular decline, reaching a minimum at the sixth leaf followed by a rise becoming steep at the ninth leaf. The value for the tenth leaf is the same as that found in all the series.

The values for the —K series are well below those of the corresponding leaves of the other series, and whereas the —N and FM values show well marked maxima the —K shows a minimum during the life-cycle.

The total sugars are therefore characterized by an increasing divergence up to the sixth or seventh leaf, and then a rapid convergence to the tenth leaf. In both total sugars and reducing sugars the original and final values are almost the same. The effect of manurial deficiency is not apparent in the early stages for reducing sugars and not very marked for total sugars, but the differences develop during the period of maximum vegetative growth (first six weeks) and gradually disappear during the time of ear formation and stem elongation.

#### *Changes in the leaf during senescence.*

The picture presented in Figs. 1 and 2 is seen even more clearly in Figs. 3 and 4 in which the sugar content at various stages of senescence of

the leaves is given. All available data are included here, successive estimations of any one leaf occurring roughly at weekly intervals. The figures above the columns are the sample numbers; leaves of any one sample number were removed at the same time (Table II).

In the FM series (Fig. 3) the general level of the values for reducing sugar confirm those already seen in Fig. 1, but the maximum value in that series at the eighth leaf is now seen to be relatively high. The whole series shows as before a slow fall in sugar content to the fourth leaf, followed by a rise to the seventh leaf, after which no great change in the general level occurs. With advancing age of leaf the changes are somewhat irregular, the first leaf showing a decline in sugar content whereas the next two leaves show little change at the two stages analysed. Larger differences are seen in the fifth leaf where a tendency for hexose to increase becomes apparent; this tendency is much more clearly seen in the later leaves where a rapid increase in hexose content accompanies senescence.

The values for the seventh and eighth leaves are somewhat erratic, but since good agreement was found between replicate leaves analysed they do not appear to be due to sampling errors (cf. Table VIII). The total sugar content of the FM series (Fig. 4) also shows rather irregular changes with leaf age. The first leaf shows a decline in sugar content and this is followed by a rise in the succeeding leaves up to the fifth leaf. The last two leaves also show a marked rise with age. As in the data of reducing sugar, the values in the seventh and eighth leaves are erratic, but here also good agreement was found between replicates (cf. Table VIII).

The reducing sugar values for the —N leaves confirm those of Fig. 1. A fall in the average sugar content from the first leaf, reaching a minimum in the third leaf, is followed by a steady rise to the tenth leaf. The hexose content in general increases with advancing age of leaves, the first two leaves, however, show a slight decrease. The data of total sugar for the same series show a regular rise in sugar content reaching a maximum in the sixth leaf, followed by a fall to the tenth leaf. In general, total sugar content increases with age.

The data of reducing sugar of the —P leaves confirm those of Fig. 1. The values show a minimum at the third and fourth leaves, attaining high values in the sixth leaf. These high values occur up to the eighth leaf, after which a fall in reducing sugars takes place. The first two leaves show a fall in hexose content with age, whereas all subsequent leaves which were sampled on more than one occasion show a large increase. The total sugar content of leaves of the same series remains fairly constant throughout, only minor changes occurring which do not appear to be related to leaf age.

The reducing sugar values for the —K series show a decrease from the first to the second leaf, after which only a slow change is seen as in Fig. 1.

Senescence has apparently little effect on the hexose content. Except for minor fluctuations the content of total sugars in the -K series remains remarkably steady and uniformly lower than in the other series throughout the whole life-cycle. The impression gained from Fig. 2 is confirmed by

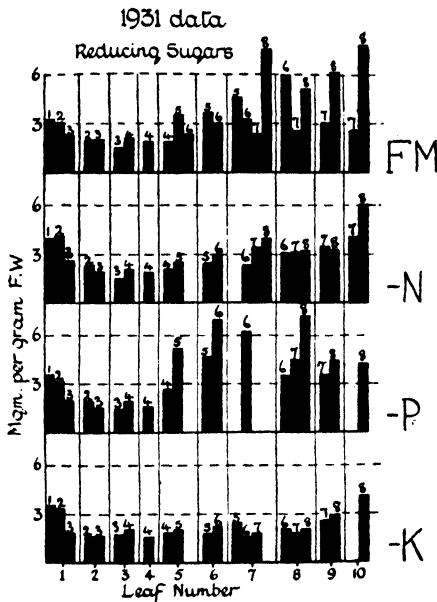


FIG. 3.

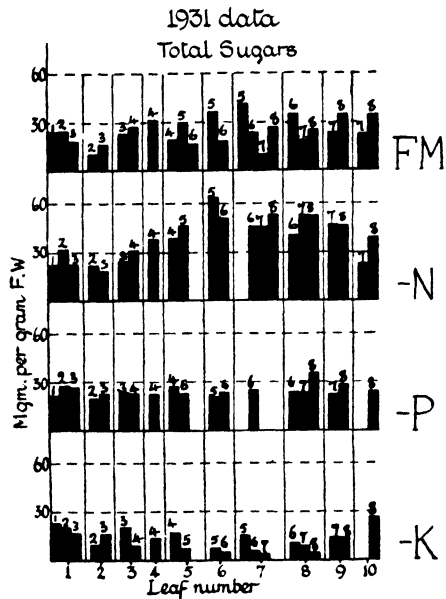


FIG. 4.

FIG. 3. Reducing sugar content (mg. hexose per gramme fresh wt.) at various stages of senescence of successive leaves on the main axis in the four manurial series. Numerals above histograms indicate sample numbers (cf. Table III).

FIG. 4. Total sugar content (mg. hexose per gramme fresh wt.) at time of full expansion of successive leaves on the main axis in the four manurial series. Numerals above histograms indicate sample numbers (cf. Table III).

the values given in Fig. 4. There appears to be a marked fall in total sugar content with advancing age of leaf.

The picture presented in Figs. 1 to 4 shows characteristic effects of nutrient deficiencies. In reducing sugar no great differences between the series are apparent up to the fourth leaf, everywhere a decline occurring with a minimum at this leaf. At the fifth leaf however a rapid rise in hexose content characterizes phosphorus deficiency, and high values are maintained to the eighth leaf.

In the FM series the rise in hexose is much slower, the eighth leaf showing a high value at the first time of sampling. The -N plants show an even slower rise in hexose content than the FM, only the tenth leaf attaining values comparable to those in the FM and -P series.

The -K plants show throughout low values for reducing sugars, no



considerable rise occurring until the ninth and tenth leaves. In contrast with this the effect of manurial deficiency on the total sugar content is seen at an earlier stage, only in the first leaf are the values in all series the same. It is now the —N series which shows a rapid increase in total sugar, reaching a maximum at the sixth leaf (Fig. 4) which is maintained until the eighth leaf. The values for the FM series show much less variation, a rise in sugar content being slower and the maximum not very well marked.

TABLE III.

*Meteorological Data of Sampling Days.*

Sample Number.	Date.	Maximum temp. ° F.	Time of sampling.	Sunrise.	Hours of sunshine up to time of sampling.
1931.					
1	May 29	62.2	12 noon	4.52	5.0
2	June 8	65.6	1.30 p.m.	4.45	5.1
3	„ 12	69.2	3 p.m.	4.44	4.0
4	„ 22	72.2	2 p.m.	4.42	5.2
5	„ 29	65.5	2 p.m.	4.45	9.4
6	July 6	66.0	3.30 p.m.	4.50	5.1
7	„ 17	62.3	5 p.m.	5.1	0.6
8	„ 22	69.4	3 p.m.	5.8	4.5
1932.					
1	June 3	60.2	4 p.m.	4.48	6.3
2	„ 11	73.8	12 noon	4.42	2.8
3	„ 15	63.3	3 p.m.	4.43	7.2
4	„ 18	66.1	11.30 a.m.	4.42	4.3
5	„ 20	55.2	4 p.m.	4.42	0.0
6	„ 25	70.0	11.30 a.m.	4.45	2.8
7	July 1	68.3	12 noon	4.46	2.6
8	„ 8	73.7	3.30 p.m.	4.52	7.2
9	„ 21	64.3	12 noon	5.6	3.4

The —P series shows almost uniform values throughout, whereas potassium starvation is characterized by extremely low values. The tenth leaf in all series show identical values for total sugar. Starch estimations were not made in these experiments. The amounts of starch present as shown by the iodine test were very small, and not sufficient for estimation. It was however noted that variations in starch content appeared between the manurial series, the order of decreasing starch being —N, FM, —P, —K. It seems very improbable that the differences in sugar content between the manurial series were seriously affected by the starch:sugar ratio. The decreased order of starch content was identical among the manurial series with the order of total sugar content.

It has been mentioned above that both reducing and total sugars show irregular values in the seventh and eighth leaves on the various occasions at which they were sampled. From the manner in which the experiment was performed variations in sugar content are liable to occur owing to the

difference in weather conditions obtaining on the days on which the samples were taken. For this reason the effect of increasing senescence in individual leaves cannot be sharply distinguished from mere fluctuations due to variation in assimilation rate prior to sampling. That the effect of the weather conditions is reflected in the sugar values obtained is suggested for the values for the seventh and eighth leaves of the FM series in both reducing and total sugars. Referring to Table III, it will be seen that on the occasion of the fifth sample (June 29) the weather was exceptionally favourable, and high values for all leaves sampled on that day are shown in Fig. 4 in the FM series. At the time of the seventh sample (July 17) the weather conditions were exceptionally poor, and this is reflected in the low values for the FM obtained on that day. The intermediate values for the sixth and eighth leaves correspond with intermediate values for the hours of bright sunshine on these two occasions. These differences are not so apparent in the deficiency series.

The varying behaviour of the individual leaves in the different series, i.e. the difference in time at which maxima and minima occur, constitutes interaction effects between manurial treatment and leaf number; reference to the section on the statistical analysis of the data show that these effects are highly significant, with a probability value much greater than 100:1. ( $z = 1.125$ , 1 per cent. point, 0.40 for reducing sugars;  $z = 1.15$ , 1 per cent. point, 0.40 for total sugars).

*Reducing sugars. 1932 data.*

The data for 1932 are presented in Table IV and graphically in Figs. 5 to 8. In Fig. 5 values for reducing sugars on the first occasion of sampling of the leaves are given. The values for the first sample show considerable variations among the treatments, but it will be noted that the first leaf was not sampled until the time at which the second leaf was mature and therefore they were already somewhat senescent.

The values for the second leaf are similar in all manurial treatments, but in subsequent leaves differences begin to appear. The FM plants from the second leaf onwards show a steady decline reaching a minimum at the seventh leaf, after which a very rapid rise in hexose occurs with a very high maximum in the last leaf.

In the -N series after the second leaf the values remain nearly constant until the seventh leaf, after which in this series also a rapid increase in hexose occurs.

The -P series shows from the second leaf onwards a slow increase to the fifth leaf, interrupted by a fall in the sixth leaf (noted as immature at time of sampling) and a rise to a maximum in the eighth leaf. The ninth leaf again shows a lower value approaching that found in the FM series. Unfortunately, no further leaves were sampled in this series.

TABLE IV. 1932 Data.  
*Sugar Content of Leaves at Emergence and at Varying Stages of Senescence, expressed as Mg. Hexose Per Gm. Fresh Weight, and the Ratio of Sucrose to Reducing Sugars, in the Four Treatments Studied. Each Entry is the Mean of Two or More Replicate Samples.*

Leaf No.	Sample No.	Fully manured.			Nitrogen deficient.			Sucrose.	
		Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.
1	1	4.89	52.42	57.31	10.72	56.77	61.77	11.35	—
2	1	3.12	28.50	31.62	9.13	28.86	31.68	10.23	—
	2	3.68	35.96	39.64	9.77	35.54	39.27	9.53	—
	3	3.67	41.56	45.23	11.32	40.13	43.86	10.76	—
3	2	2.31	11.59	23.90	5.02	19.49	22.16	7.30	—
	3	3.05	41.70	44.75	13.67	42.09	45.20	13.53	—
	5	3.09	36.53	39.62	11.82	41.21	45.75	9.08	—
	7	—	—	—	—	—	—	—	—
4	3	2.42	31.05	33.47	12.83	33.02	35.57	12.95	—
	5	2.64	22.80	25.44	8.64	34.65	37.98	10.41	—
	7	—	—	—	—	45.79	50.24	10.29	—
	8	—	—	—	—	—	—	—	—
5	4	1.96	18.57	20.53	9.47	—	—	—	—
	5	2.14	21.90	24.04	10.23	24.77	27.28	9.87	—
	6	2.23	11.58	13.81	5.19	—	—	—	—
	7	1.76	8.84	10.60	5.02	39.91	42.82	13.71	—
	8	3.38	12.55	15.93	3.71	48.04	54.77	7.14	—
6	6	1.73	12.89	14.62	7.45	—	—	—	—
	7	1.53	11.59	13.12	2.83	40.77	43.60	14.41	—
	8	3.24	20.37	23.61	3.51	69.12	72.63	19.69	—
7	7	1.56	12.61	14.17	8.08	37.76	40.74	12.67	—
	8	3.10	20.50	23.60	6.61	63.45	66.64	19.89	—
	9	—	—	—	—	—	—	—	—
8	7	2.40	16.76	19.16	6.98	—	—	—	—
	8	3.62	26.84	30.46	7.41	50.75	54.40	13.90	—
	9	—	—	—	5.34	38.93	44.27	7.29	—
9	8	5.48	41.66	47.14	7.60	—	—	—	—
	9	19.73	26.70	46.43	1.35	45.04	51.77	6.69	—
10	9	13.98	33.60	49.58	6.73	—	—	—	—
	9	20.68	41.63	62.31	13.24	38.72	51.96	2.92	—
11	9	—	—	—	—	—	—	—	—

TABLE IV (continued).

Leaf No.	Sample No.	Phosphorus deficient.			Potassium deficient.			Sucrose.	
		Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.	Total sugars.	Reducing sugars.	Sucrose.
1	1	6.65	33.38	40.03	4.18	29.47	33.65	7.05	—
2	1	2.95	18.02	20.97	2.96	27.65	30.61	9.34	—
2	2	4.39	22.70	27.09	2.95	11.41	14.36	3.87	—
3	3	—	—	—	2.67	14.37	17.04	5.76	—
3	2	3.00	12.62	15.62	2.18	13.71	15.89	6.29	—
3	3	4.52	24.19	28.71	2.57	29.93	32.50	11.64	—
5	5	4.75	22.05	26.80	2.80	13.31	16.11	4.75	—
7	7	3.66	12.57	16.23	—	—	—	—	—
4	3	—	—	—	2.42	31.30	34.94	12.93	—
5	5	3.51	18.46	21.97	2.15	12.76	14.91	5.93	—
7	7	3.97	20.37	24.34	—	—	—	—	—
8	8	5.65	28.57	34.22	—	—	—	—	—
5	4	—	—	—	2.16	16.13	18.29	7.47	—
5	5	—	—	—	1.80	15.01	16.81	8.34	—
6	6	—	—	—	2.41	8.30	10.71	3.44	—
7	7	3.63	24.44	28.07	2.40	3.25	5.65	1.35	—
8	8	6.39	24.35	30.74	—	—	—	—	—
6	6	—	—	—	2.11	9.19	11.30	4.36	—
7	7	2.42	15.87	18.29	1.71	4.01	5.72	2.35	—
8	8	3.75	19.60	23.35	—	—	—	—	—
7	7	—	—	—	1.48	6.92	8.40	4.67	—
8	8	3.13	25.77	28.90	1.65	2.59	4.24	1.57	—
9	9	10.43	32.67	43.10	—	—	—	—	—
8	7	—	—	—	—	—	—	—	—
8	8	—	—	—	1.86	4.28	6.14	2.30	—
9	9	7.28	29.11	36.39	—	—	—	—	—
9	8	—	—	—	2.01	11.01	13.02	5.48	—
10	9	4.74	23.48	28.22	—	—	—	—	—
11	9	—	—	—	4.63	10.33	14.96	2.23	—
		—	—	—	12.37	37.25	49.62	3.01	—

The  $-K$  series follows very closely the control series, but the rise in hexose is here delayed to the ninth leaf, nor is the final value as high as that of the control. In general, up to the eighth leaf for the  $-P$  plants values are considerably higher than those of the other series. The  $-N$

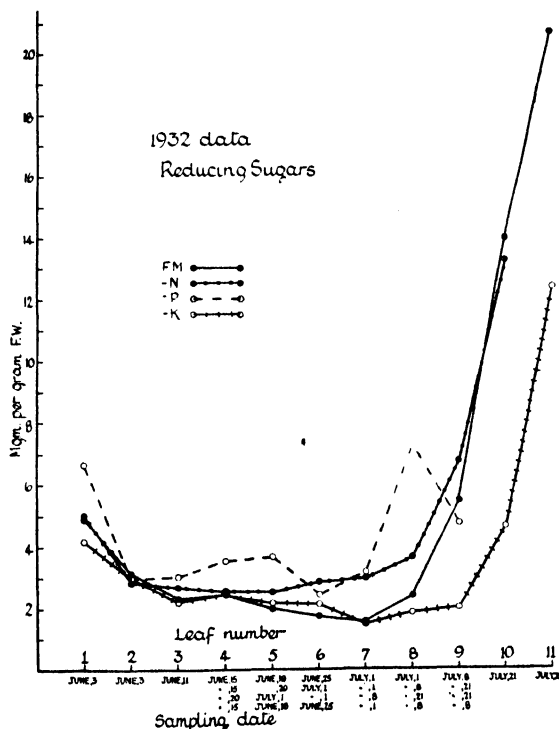


FIG. 5. Reducing sugar content (mg. hexose per gramme fresh wt.) at time of full expansion of successive leaves on the main axis in the four manurial series. Sampling dates as indicated. Manures applied in three fortnightly doses after germination.

series shows values gradually diverging from the controls, the difference in favour of the former reaches a maximum at the seventh leaf, after which the FM series approach and finally exceed them. The  $-K$  values closely resemble those of the control; only in the last four leaves do they fall below.

#### Total sugars. 1932 data.

In Fig. 6 the values for the total sugars on the first occasion of sampling are given. Here also large differences between the treatments are seen at the time of the first leaf, the  $-K$  and  $-P$  plants showing low values. Up to the third leaf the values in all series fall, the  $-K$  and  $-P$  series reaching the same value in the third leaf, and are below those of the  $-N$  and control which up to this point have the same values. In the fourth leaf a rise occurs in all the series, and with the exception of the  $-P$

series the values are the same. The level of the sugar in all leaves up to this point shows a marked relationship with the assimilation conditions obtaining at the time of sampling, the values for bright sunshine on the occasion of these three samples being 6.3, 2.8, and 7.2 hours respectively

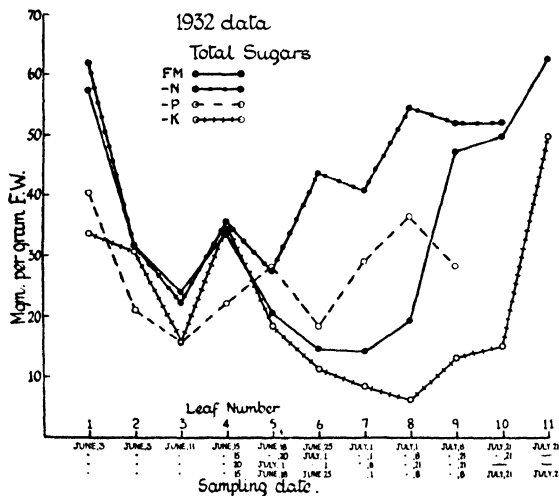


FIG. 6. Total sugar content (mg. hexose per gramme fresh wt.) at time of full expansion of successive leaves on the main axis in the four manurial series. Sampling date as indicated. Manures applied in three fortnightly doses after germination.

(Table III). The fourth leaf in the  $-P$  series was not sampled until June 20, on which day no sunshine was recorded; this may account for the low value in this series on that day. From the fourth leaf onwards marked differences due to manurial treatment appear. The FM series shows a rapid fall reaching a minimum at the seventh leaf with a subsequent rapid rise to a maximum in the last leaf, the final value being nearly the same as that of the first leaf.

The  $-N$  series shows steady rise after the fourth leaf, a low value alone occurring in the fifth leaf, which again may be referred to the adverse weather conditions on June 20.

The  $-P$  series shows a much more uniform sugar content, no consistent tendency to a rise or fall being evident.

The  $-K$  values fall rapidly from the fourth leaf, reaching a minimum in the eighth leaf, with a subsequent rise which is rapid from the tenth to the eleventh leaf.

The effect of manurial treatments on the total sugar content is characterized by very high values in the  $-N$  series, very low values in the  $-K$  series, and intermediate values in the  $-P$  series. The FM series shows values intermediate between the  $-P$  and  $-K$  series, with finally a very rapid rise from the eighth to the eleventh leaf.

*Changes in the leaf during senescence.*

The general relationships seen in Figs. 5 and 6 are even more evident in the data presented in Figs. 7 and 8, which show the effect of senescence.

In the data of reducing sugar there is a tendency in all the series, with the exception of —K, for an increase with advancing senescence of the leaves. If the values for successive leaves sampled at any one time are examined it is seen that in the FM series the leaves produced earlier have a higher sugar content than the later formed ones on a particular sampling date. This effect becomes less marked with the advance of the sampling date, so that of the leaves examined at the seventh sample the eighth leaf has the highest sugar content. On the occasion of the eighth sample values for all leaves from the ninth to the fifth are considerably higher than at the seventh sampling. This may be due to the very favourable weather conditions on that day (Table III), though here again the earlier leaves show a lower sugar value than the ninth leaf, which is younger at that date.

Between the eighth and ninth samples a great increase in reducing sugar occurs which affects the ninth as well as the two subsequent leaves. The increase in reducing sugar with advancing age is seen clearly in the —N and —P series whether values for individual leaves at different sampling dates are compared or series of successive leaves sampled on any one day.

In the —K series there is no evidence of increasing hexose content with advancing senescence of the leaf.

The general features of the data for total sugars presented in Fig. 8 confirm conclusions reached from the data in Fig. 6. In the FM and —N series there is considerable evidence of an increasing sugar content with increasing age of the leaf; here again it is difficult to separate the effects of the weather conditions on the day of sampling. In these two series the high values obtained in all leaves sampled on July 8 (eighth sample) may very well be due to the favourable weather conditions on that day. This conclusion is, however, not supported by the much lower level noted at the third sample when the weather conditions were equally favourable. Again, the values obtained at the fifth sample are in agreement to some extent with the average sunshine on that day.

In the —P series the effect of senescence is much less marked, whereas in the —K series there is considerable evidence of a fall in total sugar content with advancing age of the leaves.

*Comparison of 1931 and 1932 data.*

Comparison of the figures presented brings out very remarkable differences in the relations of sugar content within any one treatment in the two years and between treatments also. In 1931 the minimum in reducing sugars showing in the third leaf is of short duration only, at most extending to the time of full development of the fourth leaf, at which point

the large manurial differences appear and maxima are rapidly attained in the FM and -P series.

In 1932 minimal values extend over a long period. In the FM series a rapid rise begins only at the seventh leaf, and in the -P series instead

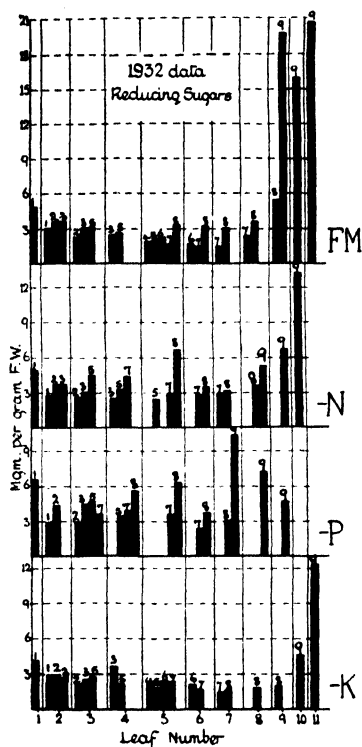


FIG. 7.

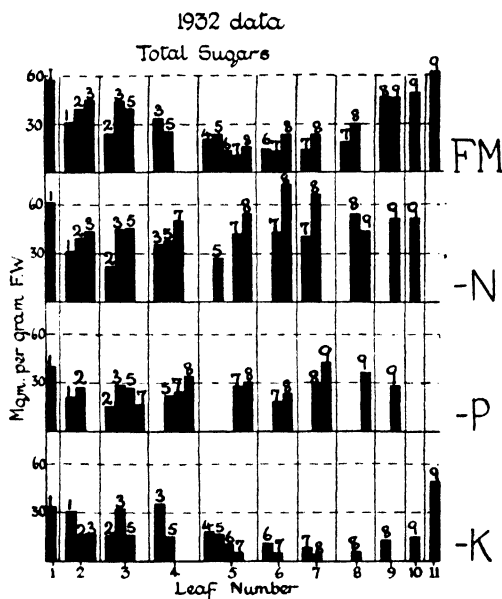


FIG. 8.

FIG. 7. Reducing sugar content (mg. hexose per gramme fresh wt.) at various stages of senescence of successive leaves on the main axis in the four manurial series. Numerals above histograms indicate sample number (cf. Table III). Manures applied in three fortnightly doses after germination.

FIG. 8. Total sugar content (mg. hexose per gramme fresh wt.) at various stages of senescence of successive leaves on the main axis in the four manurial series. Numerals above histograms indicate sample number (cf. Table III). Manures applied in three fortnightly doses after germination.

of a very rapid increase of reducing sugars only a very slow increase is seen. Again, the values for the last leaf are very much higher in 1932; this effect is seen in the FM, -N, and -K series.

In the values for total sugar equally striking differences in the two years appear, and in general the kind of differences seen resembles those of the reducing sugars. Thus, whereas in 1931 manurial differences become very apparent after the third leaf, in 1932 the appearance is delayed till after the fifth leaf. Again, well-marked maxima appear in the FM and -N series in 1931; the maximum is not attained in 1932 until the last leaf of the FM and -N series.



The results for the two years are summarized in Table V, in which values for free reducing sugar (R. S.) and total sugar (T. S.) are given. The average values for leaves at full expansion are the weighted means, due regard being taken of the number of replicates available in each sample. The average for all values included the different stages of senescence of the successive leaves: the values given are the averages of the entries in Tables II and IV. The values for 1932 include only the first nine leaves

TABLE V.

*Average Values of Sugar Content (R. S. = Reducing Sugar, T. S. = Total Sugar) in the different Manurial Treatments for the Years 1931 and 1932.*

		FM		-N		-P		-K	
		R. S.	T. S.	R. S.	T. S.	R. S.	T. S.	R. S.	T. S.
Average for leaves at emergence	1931	3.14	27.4	2.80	36.8	3.35	22.8	2.33	15.6
	1932	2.68	27.4	3.39	39.2	4.24	26.5	2.35	19.3
	(9 leaves)								
Average of all values	1931	3.50	26.8	2.92	36.9	3.74	23.1	2.24	15.8
	1932	3.60	28.6	3.82	45.6	4.71	27.4	2.34	16.3
	(9 leaves)								

as later leaves were not sampled in the -P series. The resemblance of the values in the two years is striking, and shows that the characteristic effects noted above for the various deficiencies are equally valid in the two years.

Statistical analysis which is presented in a later section has shown that in both years the effects of the treatment and their interaction with leaf number are very highly significant; this leaves no doubt that the form of the curves obtained in the two years are really different. The only possible conclusion is that these differences are to be attributed to the different method of applying manures in the two years.

#### *Relation of sucrose to reducing sugars.*

A cursory examination of the graphs already presented shows that with varying treatment and in the successive leaves of the plants the sucrose and hexose content of the leaves vary to a large extent independently. The ratio of sucrose to reducing sugars has therefore been calculated, and the values are given in Tables II and III. The same data for the two years are presented graphically in Figs. 9 and 10.

It is seen that in this respect also the results obtained in the two years differ very considerably. Dealing first with the 1931 data, in the FM series the value of the ratio rises to a maximum at the fourth leaf, after which there is a rapid fall to the fifth leaf, and a further slow fall is apparent up to the tenth leaf. There is considerable evidence in the later leaves of a

fall in the ratio with advancing senescence of individual leaves; the highest value of the ratio obtained is 15.71 in the fourth leaf.

The -N series shows a steady rise, reaching in the sixth leaf a higher maximum (24.82) than that of the FM series. After this the values

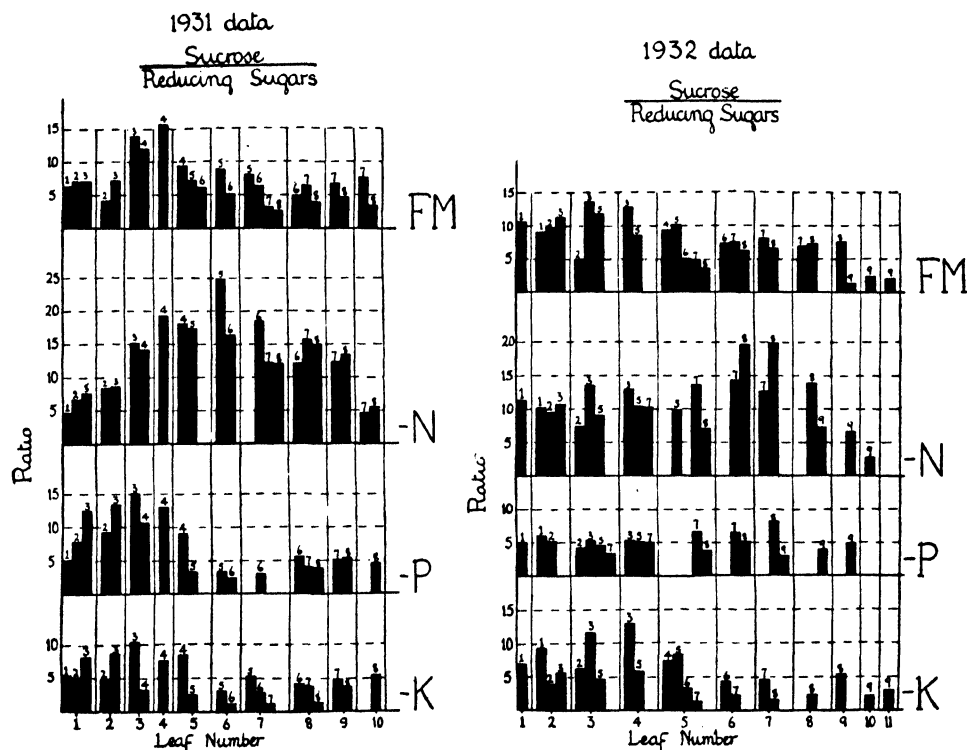


FIG. 9.

FIG. 10.

FIG. 9. The ratio of sucrose to free hexose content at various stages of senescence in successive leaves of the main axis in the four manurial series. Figures above the histograms indicate sample number (cf. Table III).

FIG. 10. The ratio of sucrose to free hexose content at various stages of senescence in successive leaves of the main axis in the four manurial series. Figures above the histograms indicate sample number (cf. Table III).

decline fairly uniformly and reach a low level in the last leaf. Again there is an indication in some of the later leaves of a fall in the value of the ratio with the age of the leaf.

The -P series shows a high value of the ratio up to the fourth leaf, with a maximum at the third leaf of 15.2, after which the value rapidly sinks to a very low minimum at the sixth leaf, with a subsequent slight recovery in the later leaves. Advancing age after the third leaf has no marked effect; the fifth, sixth, and eighth leaves show some fall.

The -K series shows uniformly lower values than the fully manured. An ill-defined maximum (10.35) appears at the third leaf, but it is not

until the sixth leaf that the values fall markedly, after which a rise occurs and the values remain fairly constant to the last leaf. From the third leaf there is marked evidence of a fall in the value of the ratio with increasing senescence of the leaf.

In all cases there is in the first two leaves evidence of a rise in the value of the ratio with increasing age of the leaf. This is without doubt due to a fall in the values of reducing sugars at that time, the total sugars remaining more nearly constant.

The results for 1932 show the following features: the FM series shows a maximum (13.67) at the third leaf, after which the value falls to the sixth leaf, remains fairly steady to the eighth, and eventually reaches very low values in the last two leaves. There is some evidence of a fall in the value with advanced senescence.

In the —N set the values are very similar to those of the FM in the first four leaves, but in this series a rise now occurs reaching a maximum value (19.69) in the seventh leaf, and this is followed by a sharp fall to the last leaf. The evidence of change with age is conflicting.

The —P series shows from the beginning values considerably below the FM and —N series. Up to the fourth leaf no change in the ratio occurs, but after this a slow rise to an ill-defined maximum at the seventh leaf (8.23) appears, the value then returning to the original level. There is some evidence of a fall of the ratio with advancing age.

The —K values begin at the first leaf considerably below those of the control series, but subsequently rise, reaching a maximum (12.93) at the fourth leaf very similar to that in the FM; after this the values decline, reaching low levels towards the end. There is clear evidence of a decline in the ratio with advancing age of individual leaves.

Comparing the series for the two years certain similarities appear, the highest ratios were obtained in both cases in the —N series, the value of the maximum being somewhat lower in 1932, but in both cases high values are found in the sixth leaf. The main difference in the appearance of the curves in this series is due to the higher values in the early leaves of the 1932 plants. A similar difference is seen in the FM series; here also the first two leaves of the 1932 plants have considerably higher values than those of the 1931 plants. The maximum is also lower, but from the fifth to the ninth leaves the values in the two years are in general similar, the tenth leaf shows much lower values in 1932.

In the —P series the outstanding differences arise from the fact that in contrast with the FM and —N series the values in 1932 are much lower than those of 1931 up to the fourth leaf; after this point, up to the seventh leaf, an increase in the value of the ratio occurs, though even in 1932 the values remain rather low.

The —K series resemble each other more closely in the two years

than any of the other series. Higher values occur in the first four leaves, after which a decline takes place, and low values distinguish the later part of the cycle.

THE ERRORS OF THE METHOD OF SUGAR ANALYSIS.

Since the work here reported was completed, further investigations by Van der Plank (46) have shown that there are serious sources of uncertainty

TABLE VI.

*Loss of Sugar from Solutions of Different Concentrations on Treatment with Charcoal.*

Mg. invert sugar per 100 cc.	Mg. invert sugar in 5 cc.	Percentage decrease in invert sugar content.
Approx.	Found.	
20	(a) 0.943 (b) 0.988	4.5
10	(a) 0.465 (b) 0.493	5.7
5	(a) 0.232 (b) 0.248	6.5
2.5	(a) 0.120 (b) 0.130	7.7
1.25	(a) 0.062 (b) 0.068	8.8

(a) = charcoal treated ; (b) = untreated.

involved in the estimates of sugar due both to the method of clarification used and to the use of the ferricyanide reagent. Van der Plank has shown that standard basic lead acetate does not effect complete removal of substances other than sugar which reduce the ferricyanide reagent. In the mangold leaf errors as large as 30 per cent. may thus occur in the estimate of free-reducing sugars. Further, fructose is lost from the solution during lead clarification and an excess of lead tends to dissolve its own precipitate, liberating again the interfering substances. Incomplete clarification therefore leads to over-estimation of reducing sugars, but this effect is in part counterbalanced by removal of fructose from the solution. Ferricyanide also was found to be inferior to copper as an oxidizing agent owing to its greater sensitiveness to reducing substances other than sugar which may be present ; it would therefore tend to an over-estimation of free-reducing sugars. For the purpose of comparison of the treatments this source of error would not reduce to any large extent the value of the results unless the content of reducing substances other than sugar was itself dependent on manurial differences. Some evidence on this point can be presented. In 1932, in addition to lead clarification, a further treatment of the solutions with charcoal (Suchar), in amounts of 60 mg. charcoal to 25 cc. of the solution, was employed, the solution containing charcoal being boiled for

one minute and filtered. The loss of sugar to be expected by this treatment may be assessed from the data in Table VI above. The entries in the table show the removal of invert sugar from different concentrations stated as mg. hexose per 100 c.c. The percentage of sugar removed is also given. The solutions were selected so as to cover the range of sugar concentrations actually encountered in these experiments. Even with the the lowest concentration only small sugar losses are found; yet the fall after charcoal treatment in the estimate of apparent sugar in plant extracts using ferricyanide is known to be considerable. This loss must be due to removal of substances other than sugar.

Duplicate analyses of solutions cleared and uncleared with charcoal subsequent to lead clarification were made in 1932 for every sample taken. From these data the regression equations have been calculated for the four treatments separately. For this purpose the very high values of reducing sugars obtained from the last sample were excluded.

The regression coefficients are as follows:

$$\text{FM, } y = 0.6914 x + 0.0029$$

$$-N, y = 0.6390 x + 0.0067$$

$$-P, y = 0.6506 x + 0.0107$$

$$-K, y = 0.5733 x + 0.0071$$

where  $y$  is the value for the sugar content of the solution treated with charcoal and  $x$  for that of the untreated.

It is seen that the regression coefficient differs little between the treatments and that the regression lines pass very nearly through the origin. It is further apparent that the values of reducing substances other than sugar for the various manurial treatments as equivalent hexose were:—FM 31 %, —N 36 %, —P 35 %, —K 43 %. The —N, —P series differ very little from the FM., only the —K series gives appreciably higher values. It will be noted, however, that the tendency has been to overestimate reducing sugar in the deficient series as compared with that of the FM by the following percentage amounts, —N 7.5 %, —P 5.9 %, —K 17 %. If correction were made for this tendency the value for —N, —P would be brought nearer to that of the controls, while in —K series the difference from the control series would be augmented. The very high values for reducing sugar in the —P series cannot be accounted for on this basis.

In view of the consideration presented above, the estimate of reducing sugars by the method adopted may be accepted as measuring real differences between manurial treatments.

As far as total sugars are concerned the results are much more trustworthy. Van der Plank has shown that in the case of the mangold leaf clarification with lead has no effect on the estimation of sucrose by the Harding and Downs copper reagent, but ferricyanide overestimates sucrose

slightly as compared with copper. No serious additional errors are introduced by acid inversion, as has been shown by a comparison with invertase hydrolysis. Total sugars however are subject to the estimate of error of reducing sugars, though here the percentage error is necessarily much smaller. Some evidence of the magnitude of the errors in determining total sugars is given in the following table; the entries in the table refer to the analysis of extracts of leaves sampled at different times throughout the life cycle of the plant.

TABLE VII.

*Comparison in Lead Clarified Solutions of the Reducing Power estimated by both Copper and Ferricyanide (Mg. Hexose Per Gm. f. w.).*

Estimation by ferricyanide.	Estimation by copper.	Difference.
30.8	25.4	5.4
22.4	19.1	3.3
11.7	7.5	4.2
19.8	14.6	5.2
21.6	17.3	4.3
23.1	17.3	3.8

It is seen that the differences are not proportional to the amount of sugar present, but on the contrary tend to a constant value. It appears that ferricyanide tends to overestimate by a constant amount the total sugars present. In the graphs showing the variation in total sugars the general level is thus too high, the form of the curves would however be unaffected by the appropriate correction.

It has recently been demonstrated that fructosans in some circumstances occur in the leaves of the barley plant as well as in the stalks and ears. Thus, Yemm (50) deduced their presence as the result of sugar analyses; moreover, fructosans have been isolated from barley leaves by Archbold in this laboratory (1). As far as is known at present, it appears that these compounds tend to accumulate only in the later leaves. It is doubtful whether the presence of fructosans has much affected the sugar values reported in this investigation.

#### THE ERRORS OF THE EXPERIMENT AND THE STATISTICAL ANALYSIS OF THE DATA.

It was planned at the outset to obtain three replicates for each leaf on each sampling occasion, but owing to shortage of material in the case of senescent leaves and the occasional accidental loss in a few cases of the samples during analysis, for some leaves only two replicate values were available. To obtain a symmetrical table for the purpose of an analysis of variance two replicates were therefore used throughout. In all cases in which three replicates of the same leaf were available, two were chosen by

random selection. The analysis of variance was carried out on the data for reducing sugars, total sugars, and the ratio of sucrose to reducing sugars, separately for the two years in which the experiment was performed. The

TABLE VIII.

*Four Manurial Series, 1931. Values of Sugar Content for Replicated Random Samples of Leaves 5-8 on the Main Axis.*

		R. S. free hexose.		T. S. total sugar as hexose		} mg. per gm. fresh weight.			
Leaf No.	Sample No.	FM		-N		-P		-K	
		R. S.	T. S.	R. S.	T. S.	R. S.	T. S.	R. S.	T. S.
5	4	1.87	19.4	2.46	35.9	3.42	30.3	1.87	24.2
		1.64	19.4	2.37	32.3	2.01	24.6	1.84	14.2
								1.86	13.7
	5	3.58	30.4	2.73	50.1	5.36	24.4	2.19	7.7
		3.64	25.3	2.56	45.5	5.07	21.0	2.06	6.9
				2.33	43.7	5.23	22.1	1.91	7.1
	6	2.23	15.8	—	—	—	—	—	—
		2.44	17.2						
	6	3.76	36.4	2.22	68.2	4.29	19.0	1.88	7.2
		3.86	42.7	2.60	57.0	4.92	21.9	1.93	8.3
		3.47	29.9	2.62	66.9	4.93	21.0	1.64	7.1
	6	3.32	20.4	3.14	45.3	7.39	27.4	2.37	4.1
		2.79	17.1	2.75	57.5	6.60	19.8	2.09	5.8
7	5	4.34	41.5	—	—	—	—	2.53	16.1
		4.61	41.9					2.41	15.3
		4.90	41.3					2.60	15.6
	6	2.84	22.4	2.36	46.0	6.79	23.0	1.58	6.1
		3.77	25.8	2.37	46.4	5.73	27.2	1.59	5.9
		2.44	12.0	3.37	45.0	—	—	1.62	3.7
	7	2.15	11.5	3.56	46.9			2.08	4.5
		2.03	7.4						
		7.36	27.5	3.61	50.1	—	—	—	—
	8	7.74	26.7	4.44	56.2				
8	6	5.32	32.5	3.02	42.2	3.49	22.3	2.10	7.4
		6.66	38.5	3.25	39.9	3.62	24.5	2.09	14.0
		2.95	21.8	3.30	57.3	4.47	21.7	1.84	8.7
	7	2.29	16.7	3.07	49.2	4.64	23.2	2.08	9.7
						4.55	24.9	1.63	8.1
						6.66	33.8	2.31	5.6
	8	6.04	29.0	3.45	55.6	7.84	37.4	1.85	4.2
		4.91	24.4	2.93	48.8				
		4.60	22.6	3.51	53.0				

values used for the analysis of variance were those for each successive leaf on the first occasion of sampling.

Exigencies of space will not permit a presentation of all the data, but in order to show the agreement between replicated samples of particular leaves taken at random some of the analytical data for 1931 are given. The selection of data was made to include some of the most variable figures for individual leaves at different stages of senescence (see Figs. 3 and 4 FM leaves 5-8), and to confirm the statement made above that these variations are not due to chance fluctuations. Further, the evidence for the

high values of reducing sugar in the -P series in leaves 5-8 is also included. The results are given in Table 8, and include all the analytical data for leaves 5-8 for each manurial treatment.

In 1931 the values for ten leaves were used in the statistical analysis, but in 1932 values for the first nine leaves only were used as the -P series at the time of the last sample had expanded only nine leaves whereas the other series had eleven leaves. This discrepancy was due to the slow rate of leaf production in the -P series.

The results of the analysis are given in the following table.

TABLE IX.

*1931 Data.*

	Degrees of freedom.	R. S.	T. S.	Ratio	Sucrose R. S.	1 % point ( $P = 0.01$ ).
Leaf number	9	1.883	1.307		1.105	0.544
Treatment	3	1.405	2.400		1.805	0.738
Interaction	27	1.125	1.151		0.648	0.426
Error	40	$\sigma = 0.416$ (14.6 %)	$\sigma = 3.868$ (15.2 %)	$\sigma = 3.086$ (34.1 %)		

*1932 Data.*

Leaf number	8	2.508	2.332		1.586	0.566
Treatment	3	3.550	2.749		2.511	0.744
Interaction	24	1.703	1.751		1.324	0.436
Error	36	$\sigma = 0.249$ (7.69 %)	$\sigma = 2.380$ (8.19 %)	$\sigma = 0.830$ (10.2 %)		

The data entered in the Table are the values of  $z$  with the number of degrees of freedom available for the estimation of variance. The requisite values of  $z$  for a probability  $P = 0.01$  are also given, together with the standard deviation of the random sampling error, both actual values and as percentage of the general mean.

The errors in the 1931 experiment are uniformly larger than those of the 1932 experiment and for the sugar estimations range from 7.7 per cent. to 15.2 per cent. The standard deviation of the ratios of sucrose to reducing sugars are necessarily higher, reaching 34 per cent. in 1931.

The statistical analysis shows that in both years the results for variation due to leaf number, manurial treatment, and their interaction are very highly significant, in every case the ' $z$ ' values being much greater than the 1 per cent. probability value. A comparison of the effects of all the treatments in pairs in the two years is given in Table X together with the standard error of the difference.

From the comparison of the mean values for each deficient series with those for the controls it is seen that in all cases—with the exception of phosphorus deficiency for reducing sugar content for 1931 and that for



total sugar content for 1932—these means differ significantly; the difference always exceeding twice the standard error. Compared among themselves the various deficient series differ very significantly.

TABLE X.

*Differences of Means of Treatment. Averages for Leaves at Emergence.*

Reducing sugar content.						
	FM v. -N	FM v. -P	FM v. -K			
1931	+0.341 ± 0.115	-0.210 ± 0.118	+0.808 ± 0.115			
1932	-0.706 ± 0.075	-1.554 ± 0.073	+0.338 ± 0.074			
	-N v. -P	-N v. -K	-P v. -K			
1931	-0.551 ± 0.115	+0.467 ± 0.113	+1.018 ± 0.115			
1932	-0.848 ± 0.074	+1.044 ± 0.074	+1.892 ± 0.074			
Total sugar content.						
	FM v. -N	FM v. -P	FM v. -K	-N v. -P	-N v. -K	-P v. -K
1931	-9.43 ± 1.07	4.61 ± 1.09	11.83 ± 1.07	14.04 ± 1.07	21.26 ± 1.05	7.22 ± 1.07
1932	-11.76 ± 0.72	0.94 ± 0.70	8.11 ± 0.71	12.70 ± 0.73	19.87 ± 0.73	7.17 ± 0.73

## DISCUSSION.

In the following discussion use will be made of data accumulated at various times in parallel investigations. Some of these data are unpublished, but much is incorporated in theses submitted for higher degrees, and may be consulted. The references to such work is given in the bibliography.

The sugar level in a leaf may be considered as a resultant of the following four rates: 1. The rate of photosynthesis; 2. the rate of respiration; 3. the rate of translocation; 4. the rate of utilization for the synthesis of other substances.

(1) *The rate of photosynthesis.* Earlier experiments in this Institute have given very clear evidence of the effect of manurial deficiency on the rate of photosynthesis. The evidence is based on two independent estimations of the assimilation rate: (a) from direct measurements of CO<sub>2</sub> assimilated by successive leaves attached to the plant and under normal illumination and normal CO<sub>2</sub> supply of the air (5); (b) from the analysis of dry weight increase (10, 25).

Both methods have given concordant results. The first method has shown that in both the FM and -N series there is no sign of difference in assimilation rate in the successive leaves at the time of their full emergence. Furthermore, there is no difference in assimilation rate between the FM and -N series; the mean values obtained in 1933 by direct method for assimilation rate in these two series being FM 19.4 and -N 20.1 mg. CO<sub>2</sub> per dm<sup>2</sup> per hour (5).

In the -P series assimilation rate falls after the fifth leaf, reaching a minimum at the ninth leaf with a subsequent rise.

In the —K series the assimilation rate declines in the successive leaves, reaching a minimum at the eighth leaf with a recovery afterwards.

Analysis of dry weight data has shown that in all leaves of the plant up to the period of maximum leaf area there is no difference in assimilation rate between the FM and —N series (FM 5.7, —N 6.0 mg. CO<sub>2</sub> per sq. decimetre per hour) but that subsequently, owing to the larger proportion of senescent leaves in the —N series, the net assimilation rate falls to lower values than those of the FM. The average value obtained in 1931 over the whole growth period were FM 6.5, —N 5.7 mg. CO<sub>2</sub> per sq. dec. per hour (25).

In the —P series values similar to those of the FM are obtained for the first six weeks, but they subsequently fall below those of the FM series. The values for —P series obtained in 1933 (47) were FM 10.2, —P 10.3 for the first six weeks, and FM 10.2, —P 7.7, over the whole period.

In the —K series considerably lower values throughout the life cycle were obtained. The values obtained in 1931 were as follows: FM 6.5, —K 4.3 for the whole period (25). The low values for sugar content of the leaves of the —K series are therefore directly related to the low assimilation rate in this series. The high values for the —N series cannot be due solely to the variation of assimilation rate.

Before discussing the effect of the other factors stated above the following data may be presented. From the values of assimilation rate derived from the dry weight data it is possible to calculate the concentration of sugar to be expected in the leaves, assuming that all material formed in assimilation appears as sugar and is not translocated.

Since on the average the samples were taken nine hours after sunrise the calculated values are those for this period of time. The assimilation rate has been stated above as mg. CO<sub>2</sub> per square dm. per hour, and in converting these to mg. hexose per gm. fresh weight due allowance has been made for the variation in fresh weight per sq. dm. in the different series. The following data for the relation between fresh weight and leaf area were used:

FM	1 gm. fresh weight	=	0.45 sq. dm.
—N	„ „	=	0.49 „
—P	„ „	=	0.39 „
—K	„ „	=	0.52 „

Comparison of hexose expected with that found is shown in the following table:

TABLE XI.

	FM	—N	—P	—K
Hexose expected	18.0	17.1	11.9	13.6
Hexose found <sup>1</sup>	25.2	38.9	24.1	13.7
Excess sugar	7.2	21.8	12.2	0.1

<sup>1</sup> Means of all determinations, see Table II.

In all the series, with the exception of —K series, the sugar present in the leaves is higher than can be accounted for by the mean assimilation rate. To account for these discrepancies the following considerations are put forward.

The assimilation rate used is based on the average assimilation rate over a long period. It is well known from the work of Davis, Daish, and Sawyer (6) that during the early period of growth of the mangold the sugar level in the leaf reaches a maximum at about 2 p.m., and Miller (27) has shown that the dry matter content of maize and sorghum leaves undergoes a diurnal variation, reaching a maximum in the early afternoon and then rapidly declining. The time of sampling therefore corresponded with the maximum sugar content of the leaf. Further, the period before sampling covered the morning hours, and it is by no means certain that the net gain in carbohydrate in the afternoon is as high as it is in the morning hours even under the same external conditions. The accumulation of dry material in cereal plants exposed to short days (10 hours) has been shown by Purvis (35) to be as high as that of plants exposed to the whole day (16 hours) in spite of the shorter period of assimilation. For these reasons the value of the hexose content calculated from the average assimilation rate may be too low. The amount of these discrepancies varies with the manurial treatment; the treatments being in the following descending order —N, —P, FM, —K.

The —N and —P treatments are characterized by a great reduction in meristematic activity as shown by the low tiller numbers obtained in these series. The —K treatment, on the other hand, shows an increase of meristematic activity over the FM, the maximum tiller numbers in this series being higher than in the FM series. The maximum tiller numbers in the various deficiencies as compared with FM taken as 100 are as follows (25) (47):—

—N	. . .	37
—P	. . .	53
FM	. . .	100
—K	. . .	111

The order of the treatment is therefore the same as that of the falling sugar contents and that of the falling sugar excess, but now represents a series with rising meristematic activities.

For the production of vegetative buds (tillers) sugar must be translocated, and in so far as the rate of translocation is controlled by concentration in the leaf one would expect that the higher sugar contents of the leaves would correspond with the higher tiller numbers. Since exactly the reverse relation is found it may be suggested that the high sugar content is a consequence of the low tillering. Where the tillering is low therefore, sugar will tend to accumulate in the leaves and presumably also in the whole plant.

The -N series show the effect of this sugar accumulation very markedly both in the 1931 and 1932 experiments. A preliminary fall in the early stages is, no doubt, associated with the laying down of tillers, the first visible tiller appearing at the time of full development of the third leaf. Records of tillering made by Mathur in 1931 show that maximum tiller production rate in both the FM and -K series occurred after the fifth leaf. In both these series a minimum in the sugar content occurs about this time; a similar minimum is found in 1932 also.

In the FM and -K series tillering ceases at the time of emergence of the eighth leaf; in 1932 in both these series a rise in sugar content of the leaves begins at this time. In 1931 this effect is seen in the -K series but is not apparent in the FM series.

In the -N series the maximum tiller production rate occurs after the emergence of the third leaf and no further tillers are produced after the time of the emergence of the fifth leaf. The highest sugar values in the 1931 experiment occur immediately after this, and in 1932 this point marks the beginning of rapid sugar accumulation.

In the -P series no marked sugar accumulation occurs after emergence of the fifth leaf, but in this series this marks the beginning of acute phosphorus starvation and a rapid fall in assimilation rate. It would appear therefore that the sugar level in the leaf and probably in the whole plant also is markedly affected by tiller production.

2. *The rate of respiration.* The fall in sugar level is dependent also on the respiration rate. Experiments performed by Gregory and Richards<sup>1</sup> (12) have also shown that respiration varies in the deficient series, the mean values being FM 0.50, -N 0.47, -P 0.49, and -K 0.73 mg. CO<sub>2</sub> per gm. fresh weight per hour. The very high value of respiration in the -K series is confirmed by further work by Richards (36) and Sen (41); the values in the -P series differ very little from that of the FM. The values given above are for 17° C. which is higher than the average night temperature in summer. Assuming an average night of six hours the sugar losses of the leaves by respiration would amount to FM 3.0, -N 2.8, -P 2.9, and -4.4 mg. hexose per gm. fresh weight. These are small compared with the values for the sugar content found and they cannot have much differential effect on the sugar level in the different series.

3. *The rate of translocation.* Data for the dry weight of leaves, stems, and roots of the barley plant grown under the same manurial treatments as in these experiments and sampled at fortnightly intervals, have been collected for FM, -N, and -P plants by Gregory (unpublished data), and for FM, -N, and -K plants by Mathur (25).

The ratio of the total dry weight of stems and roots to that of leaves

<sup>1</sup> The manurial deficiency in these experiments was less severe than those here employed, being one-fifth the standard amounts for N and P, and one-ninth for K.

alone should give some measure of the relative amounts of assimilation material held in the leaf or used for the formation of other young leaves, and that translocated away and used for other purposes; therefore, one would expect some relationship between the concentration of sugars in the leaf and this ratio.

The average values of this ratio, together with the average values for sugar content in the two years, are given in Table XII below. The data cover the first eight weeks from germination. The entries of the ratio of weight of stem and root to leaf in the Table are the mean values for the four fortnightly periods.

TABLE XII.

		FM	—N	—P	—K
Dry weight	$\frac{\text{stems + roots}}{\text{leaves}}$	1.35	1.60	1.75	1.02
Sugar content of leaves as mg. hexose per gm. fresh weight	{ (1931)	29.87	38.07	22.36	15.37
	{ (1932)	28.55	37.33	22.57	11.08

Comparison of the FM and —N series shows that the higher sugar content is associated with the higher translocation rate. In these cases, as stated above, the assimilation rate is the same.

The —P series shows an even higher value for translocation than the —N series, but in this case it is associated with a lower sugar content than in the FM series, this effect being due no doubt to the lower assimilation rate.

The —K series shows the lowest sugar content associated with the lowest translocation rate. These data therefore do not account to any large extent for the variation in sugar level found. It would appear that the variation in assimilation rate is more important than variation in translocation rate in determining the varying sugar level in the different treatments.

4. *The rate of utilization of sugar for synthesis.* Of the substances other than sugar formed in the leaf from carbon assimilated, by far the most important is the protein. To estimate the effect of protein synthesis on the sugar level of the leaves, intervals from the sixth to the eighth week have been examined, corresponding with the production of the sixth to the eighth leaf on the main stem of the plant, which covers the period of maximum leaf area. The values for protein nitrogen in these leaves have been determined by Richards and Templeman (37) for the same deficient series as here used, and on plants grown under the same conditions as the 1931 series. The data of protein content per gm. fresh weight calculated from Richards' data are given below. Protein has been assumed to contain 17 per cent. of nitrogen.

FM	. . .	34 mg.
—N	. . .	16 mg.
—P	. . .	27 mg.
—K	. . .	23 mg.

From the data presented above the values for sugar in the leaves (as mg. hexose per gm. fresh weight) have been calculated for the same period and are given below :

FM . . .	38 mg.
— N . . .	50 mg.
— P . . .	23 mg.
— K . . .	11 mg.

Assuming the carbon content of protein to be 53 per cent., the weight of hexose with the same carbon content as that of the protein in the different series given above is :

FM . . .	44 mg.
— N . . .	21 mg.
— P . . .	35 mg.
— K . . .	30 mg.

It is apparent therefore that with the exception of the — N series the carbon present as protein in the leaves is equivalent to considerably more hexose than corresponds with the total sugar content of the leaf.

The consumption of sugar in protein synthesis is seen from the table to be twice as great in the FM series as in the — N series.

Taking all the factors considered into account, the effects of manurial treatment are summarized in the table below. The shortcomings of the data available for this comparison are realized. All data are compared with the FM series taken as 100.

TABLE XIII.

	— N	— P	— K
	%.	%.	%.
Translocation { calculated from change of ratio }	+ 20	+ 32	— 23
Respiration } of leaves to remainder of plant }	— 7	— 3	+ 47
Assimilation (from dry weight data)	— 5	— 34	— 25
Protein synthesis (from data of leaf protein)	— 52	— 21	— 32
Sugar content of leaves (at emergence) <sup>1</sup>	+ 37	— 14	— 41
Sugar content of leaves (means of all determinations) <sup>1</sup>	+ 54	— 4.4	— 46

<sup>1</sup> See Table II.

The high level of sugar in the — N series may be attributed largely to the high assimilation rate (which is practically the same as in the FM series) and low consumption of sugar in protein synthesis. The lower values of sugar in leaves of the — P and — K series are due to the lower assimilation rate. The — K series suffers in addition great loss of sugar by protein synthesis and by respiration, and this series shows the lowest sugar concentration of all.

These results are in agreement with previous results on the effects of the meteorological conditions on the growth of the barley plant found by Gregory (10). It was there stressed that in fully-manured plants carbohydrate rarely becomes the controlling factor in growth, indicating a reserve of sugar which may be called upon when the conditions for photo-synthesis

are not adequate to supply the necessary sugar for maintaining growth at the maximum level set by the external conditions.

In the  $-K$  series, however, there is no excess of sugar, and the behaviour of these plants reflects these conditions in two respects: (1) bright sunshine is essential for the growth of potassium-deficient plants, as has been noted by other workers. Thus Russell (40) states that 'potassic fertilisers act well in cold sunless seasons'; (2) at the time of the development of the ear rapid death of the tillers takes place due, no doubt, to the demand for protein synthesis by the developing ear and elongating stem. In this connexion also the findings of Tubbs (44) on the development of mechanical tissue of the stem under these same deficiencies are explained. A very poor development in mechanical tissue in the  $-K$  series is due to lack of reserve carbohydrate, whereas the very high development of mechanical tissue in the  $-N$  series is correlated with a large excess of sugar found over that required for the purposes enumerated.

The difference in appearance of the curves for 1931 and 1932, due to the difference in application of manures, is no doubt associated with the fact that ear production was delayed in 1932.

With the development of the ear, and more particularly after fertilization, a large demand is made on the sugar reserve for protein synthesis, and the fall in values of the total sugar in 1931, leading to a rapid fall after the eighth leaf in the  $-N$  and FM series, is due to this second cycle of high protein synthesis. The rise in the  $-K$  series observed in this year may be attributed to the failure of the ears to develop, and the increased rate of assimilation noted in these plants towards the end of the growth cycle.

From the value of the ratio of sucrose to reducing sugars seen in Figs. 9 and 10, it would appear that the excess sugar which is stored in the leaves of plants of the FM and  $-N$  series is sucrose. The most characteristic effect seen there is the different behaviour of the  $-P$  plants in which the ratio of sucrose to reducing sugars in the two years is, in the later stages, much lower than in the FM plants.—This accumulation of reducing sugars presents an interesting problem. In 1931 the change in the ratio is particularly rapid between the fourth and fifth leaves, and it is interesting to note that a similar change in the nitrogen metabolism was noted by Richards and Templeman (37), who found that a sudden increase in amide content occurred at that time in the  $-P$  series alone.

Richards' data have been used to elucidate this point. It was assumed that a tendency towards equilibrium exists between the sucrose and reducing sugar, and the value of this equilibrium relation was estimated from the mean ratio throughout the growth cycle in the FM plants. Using this value, the amount of reducing sugar to be expected at any time was calculated for each sample in each series from the amount of sucrose present. The difference between the actual free hexose and the calculated free

hexose value was then compared with the amide present expressed as a percentage of the total amino nitrogen. The correlation coefficient of these values was estimated for the  $-N$ ,  $-P$ , and  $-K$  series, and gave a value of  $r = 0.432$ , with a probability value greater than  $P = 0.01$ . It appears therefore that the accumulation of reducing sugar in all series, and particularly so in the  $-P$  series, is associated with an excess of amide. The evidence points to the fact that phosphorus is essential to both sucrose formation and protein synthesis. Richards (37) has already emphasized the importance of phosphorus for protein synthesis, and it would appear that it is equally important for the synthesis of sucrose.

The effect of senescence on the sugar level of the leaves has been mentioned in another section of this paper. It is interesting to note that even in advanced stages of senescence the sugar level is still high and indeed may increase markedly as is seen in the  $-N$  series.

Only in the  $-K$  series does the sugar level consistently fall with age of the leaf.

Combes and Kohler (4) and Michel-Durand (26) have shown that a considerable loss of carbohydrate takes place with leaves of trees shed in the autumn. The latter investigator reports similar losses with the dying of the leaves of annual plants.

#### SUMMARY.

1. Barley (var. Plumage Archer) was grown in sand culture. Four manurial treatments were studied: fully manured (FM) receiving a standard dressing containing 1.5 gm. N, 1.0 gm.  $K_2O$ , 0.5 gm.  $P_2O_5$  per pot of three plants (30 lb. sand); and the deficient series ( $-N$ ,  $-P$ ,  $-K$ ) each receiving  $\frac{1}{10}$  the standard amount of the nutrient in deficit. Over 200 pot cultures were employed to supply material for analysis.

2. The experiment was performed in two seasons 1931 and 1932; the nutrients in 1931 were added in one dose at germination, but in 1932 during the first six weeks in fortnightly doses.

3. Successive leaves on the main stem were sampled when they were fully emerged and also at varying stages of senescence. The sugar content was estimated on the alcohol extract by the Hagedorn and Jensen method. The errors of the method are discussed.

4. Comparing the deficient series with the fully manured (Table V. Leaves at emergence) the following results appear:

- (a) Nitrogen deficiency has no consistent effect on free reducing sugar (1931  $-11$  per cent., 1932  $+26$  per cent.); total sugar is increased (1931  $+34$  per cent., 1932  $+43$  per cent.).
- (b) Phosphorus deficiency increases free reducing sugar (1931  $+7$  per cent., 1932  $+58$  per cent.), total sugar is less affected (1931  $+17$  per cent., 1932  $+3$  per cent.).



- (c) Potassium deficiency lowers free-reducing sugar (1931 — 26 per cent., 1932 — 13 per cent.); total sugar is also greater reduced (1931 — 43 per cent., 1932 — 30 per cent.).

These average values include the early leaves in which, before external symptoms of manurial deficiency appear, no large differences are apparent between treatments in the leaves at full emergence.

5. For free reducing sugar the following average differences between the deficient series (Table X) were obtained :

— N v. — P	1931 — 20 per cent.	— N v. — K	1931 + 17 per cent.
	1932 — 25 per cent.		1932 + 31 per cent.
— P v. — K	1931 + 30 per cent.		
	1932 + 45 per cent.		

The corresponding values for total sugar were :

— N v. — P	1931 + 38 per cent.	— N v. — K	1931 + 58 per cent.
	1932 + 32 per cent.		1932 + 52 per cent.
— P v. — K	1931 + 32 per cent.		
	1932 + 27 per cent.		

6. The variation in sugar level throughout the series of successive leaves in the two seasons is described. No consistent differences in sugar content appear until after the emergence of the third leaf, at which time tillering begins.

7. The effect of senescence of individual leaves is considered. In the earliest leaves sugar tends to diminish with advancing age of leaf but in the later leaves the opposite effect appears, the change occurring at different stages of growth in the various series. In the —K series alone does the sugar level consistently fall with the age of leaf. Variations in sugar level are shown to be related to the weather conditions prior to sampling.

8. The ratios of sucrose to free reducing sugar are presented and are shown to vary characteristically in the different series, being very high in —N, very low in —K, and intermediate in —P.

9. A statistical analysis of the data is given, and it is shown that all the effects obtained are very highly significant.

10. The high value of reducing sugar in the —P series is shown to be *related to the high ratio of amide to total amino nitrogen* in this series, a sudden increase in both values occurring at the time of emergence of the fourth leaf.

11. The relation of sugar level to the factors of carbon assimilation, translocation, protein synthesis, and meristematic activity is discussed.  
(a) High sugar content in the —N series is due to high assimilation rate, low respiration, low protein synthesis, and low meristematic activity.  
(b) The lower sugar content in the —P series (almost same as FM) is due

to the lower assimilation rate and higher translocation than in the —N series: the respiration rate is also lower, and the protein synthesis much reduced together with meristematic activity. (c) The very low sugar content in the —K series is due to very low assimilation rate, very high respiration, considerable protein synthesis, and excessive meristematic activity.

In conclusion the authors wish to acknowledge their indebtedness to Sir J. Russell for facilities for the work to be carried out at Rothamsted Experimental Station.

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# Studies in Tropical Fruits.

## I. Preliminary Observations on Some Aspects of Development, Ripening and Senescence, with Special Reference to Respiration.

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With fifteen Figures in the Text.

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## I. INTRODUCTION.

AMONG plant physiologists it has been accepted that in the development of plant organs the intensity of respiration supplies a general indication of the magnitude of metabolism. In particular, in the study of fruits, it has been found that the 'ontogenetic metabolic drift which characterizes the progress of cells from adolescence through maturity to death' (1) is represented in respiration by a curve of characteristic shape. This shows two high values separated in time: one is initial, and represents a high rate of respiration in young fruits; the other, occurring at the onset of senescence, is known as the climacteric, and is generally associated with the change in colour in ripening fruit.

The reservations which must be adopted in assessing the significance of respiration measurements have been clearly outlined by F. F. Blackman (1). Not only is the organ whose respiration is being studied a heterogeneous collection of different, though interrelated, cell and tissue units, but such units themselves exhibit a wide range in activity according to the stage they have reached in their development. As a result of this absence of uniformity in time and space, a respiration curve does not give a true picture of the metabolic activity of individual cells or tissue units, but 'tends towards being only a statistical curve of the distribution of states of greater or less activity amongst the cell population'. Nevertheless, despite the drawbacks and limitations presented by respiration studies, this method of investigating metabolic rate continues to be used and, particularly in the commercial storage of fruits, the results may on occasion be applied with considerable practical advantage. On the other hand, it will be generally admitted that in the fundamental processes involved in the development, maturation, and senescence of plant tissues, serious difficulties have to be overcome in attempting to correlate the rate of respiration at any particular point in time with the major internal changes in progress at that time. For example, so far as the writers are aware, no adequate theory has been put forward as to why a high value should be associated with the metabolism of very young fruits. Again, the phenomenon of the climacteric rise in ripening fruits is not yet fully understood, and further, with a few exceptions (16), has not been directly correlated in time with the more prominent internal changes as ascertained by biochemical studies. In some respects it is thought that the data advanced in this paper may afford new points of view in the attempt to interpret certain aspects of respiration and maturation in fruits.

Reference to contemporary literature not only reveals wide gaps in the correlation of metabolic processes with the phenomenon of respiration as generally understood, but indicates that a coherent picture in terms of anatomy, morphology, physiology, and biochemistry, of the progressive

phases of growth, organization, and maturation which culminate in the production of the ripened fruit has yet to be presented. For one of the most complete pictures, the reader's attention may be directed to the useful summary of apple investigations by Kidd (16) in which a scheme of respiration and biochemical changes during the life of one particular fruit is outlined.

Since recent studies of fruit have been almost entirely physiological in inception and treatment, what appears to be required at the present time for the greater elucidation of certain major problems is a more comprehensive outlook on the several related aspects of the subject. Such an attitude will of necessity direct attention to other aspects than those usually selected for physiological investigation and should accordingly give the conclusions a wider and more general botanical significance. It is thought that the expression of this point of view will not only materially assist in the solution of the particular problems with which the physiologist is confronted, but will also throw open for more thorough investigation what may be regarded as a central feature of the plant kingdom, namely, the physiology of developmental processes (together with the associated morphological and physiological phenomena) and the succession of changes which result in the organization of the fully developed fruit and end with the liberation of the seeds.

## II. SCOPE OF THE INVESTIGATIONS.

In these preliminary investigations, attention has been directed to:

- (i) the shape of the transpiration and respiration curves in developing, ripening, and senescent fruits;
- (ii) the internal gas concentrations obtaining during these phases;
- (iii) the correlation of the above with the organographic and tissue changes observed throughout the life of the fruit.

The reasons for undertaking these investigations are based on the following considerations. From respiration studies undertaken by other workers it has been demonstrated that the epidermis, to some extent, limits the outward passage of  $\text{CO}_2$  from the respiring tissue within, and likewise the access of oxygen to those tissues. Accordingly a number of important inferences may be drawn: (a) that internal concentrations of  $\text{CO}_2$  in excess of those found in the normal external atmosphere may be present in the network of intercellular air-spaces and in the tissues themselves; (b) that respiration, as normally assessed by measurements of the  $\text{CO}_2$  liberated through the epidermis, may not reflect the true rate of  $\text{CO}_2$  production by the aggregate of living cells at that time; (c) that changes in the rate of liberation of  $\text{CO}_2$  externally may be referable (i) to modifications in the rate or kind of metabolism, (ii) to altered resistance to the passage of gases as a result of changes in the epidermal or sub-epidermal tissues, and (iii) to changes in the capacity of tissues for retaining  $\text{CO}_2$  and other gases.



In so far as water relations are of fundamental importance throughout the life of the fruit, and as transpiration rate is conditioned by internal factors as well as by the resistance offered by the relatively impervious epidermis, measurements of water losses have also been made wherever possible.

### III. INTERNAL GAS CONCENTRATIONS OF FRUITS.

The accumulation of carbon dioxide within the tissues of plants has long been known, and from the time of Ingenhousz (1788) onwards, several investigators have published interesting, though insufficient, data on this aspect. In particular, mention may be made of the work of Gerber (5) who reported a low proportion of oxygen in the gases of fleshy fruits. In a recent work Kertesz (10) has summarized the relevant literature; reference to the several papers cited shows that the data tend to be disconnected, and in general, that the several researches were not undertaken with a view to the elucidation of metabolic processes. It seems evident, from the argument outlined in the previous section, that respiration studies, as usually conducted, should include an examination of internal gas relationships.

### IV. CHOICE OF MATERIAL AND METHODS.

In view of intensive current investigations of respiration as a means of determining the course of metabolism in fruit, and as a possible avenue of access to the more fundamental study of gas-storage phenomena, the writers were led to a detailed consideration of the internal gases of certain tropical fruits, those selected for special study being characterized by their large size when full grown and the presence of internal cavities or an extensive aeration system. It is evident that the choice of such favourable material not only greatly simplifies experimental study, but also that the results will be applicable in some degree to smaller, solid fruits whose internal gases do not so readily lend themselves to analysis by similar direct methods. On the subject of internal gas concentrations in the apple, Kidd (16) has pointed out that: 'Owing to the resistance to diffusion offered by the peel, the composition of the internal atmosphere differs considerably from that of air. Conditions can easily arise which lead, through a conjunction of low skin porosity and high respiration activity, to concentrations of carbon dioxide inside the fruit which are directly injurious. So much has been definitely shown, but so far no thorough study of the internal atmosphere in relation to the variables affecting it during growth and storage has been carried out.'

#### (a) *Respiration and transpiration measurements.*

In comprehensive respiration studies, a common method is to collect fruits at intervals through the growth period, and to construct a curve

showing the rate of respiration plotted against age. In the present studies, as a matter of convenience, rates of respiration were plotted against fresh weight; the difference between the two types of curve is thus chiefly an expression of the rate of growth for the fruit under observation. Where rate of respiration plotted against weight yields aberrant points, it is probable that these reflect, as Gustafson (7) has indicated in tomato studies, that physiological and chronological age are not necessarily synonymous.

The experimental method adopted was as follows: fruits representing as complete a size-range as desirable during development, were collected at the same time from one tree and taken expeditiously to the laboratory. A size series was then selected, fruits of anomalous symmetry being rejected. Weights and volumes were ascertained and the fruits placed in gas-tight containers of suitable dimensions. After a sufficient lapse of time, based on previous knowledge of the respiratory activity of the fruit under consideration, the amount of  $\text{CO}_2$  liberated was determined by means of the Haldane Gas Analysis Apparatus; in no case was  $\text{CO}_2$  allowed to accumulate beyond a concentration of 0.5 per cent. The rate of transpiration was ascertained by measuring the loss in weight over a given time during which the entire series of fruits had been maintained under uniform conditions.

(b) *Method of obtaining internal gas samples.*

With large fruits such as the papaw, pumpkin, water-melon, bread-fruit, granadilla, gourd, cucumber, cocoa pod, &c., no difficulty was encountered in securing samples for analysis by the Haldane apparatus. Glass tubes of suitable diameter fitted with a well-vaselined rubber washer and clipped rubber tube, were inserted into the fruit, under aseptic conditions, in a hole made by a cork-borer as soon as the plug of tissue was withdrawn. The extracted plug offered evidence of the stage of maturity as indicated by the seed and flesh condition. The tube was then attached to the gas analysis apparatus, a sample of the internal gas was withdrawn and the amount of carbon dioxide and oxygen determined in the usual way. Even in fruit of the size of a fully-grown cucumber it was possible to withdraw two successive 10 cc. samples without creating an undesirable reduced pressure or leakage at the tube. With larger fruits such as the papaw, with an internal cavity varying from 250–1250 cc., sampling presented no difficulty whatsoever. With smaller fruits, where reduced pressures were encountered on withdrawing relatively small gas samples, it was found more convenient to place the passage to the interior of the fruit in a mercury trough as soon as the cork-borer was withdrawn, and insert a bent tube leading to the apparatus.

The data obtained show that considerable internal concentrations of carbon dioxide are the rule in tropical fruits. In green fruits, i.e. those which have not yet reached the climacteric, the results so far obtained

indicate that the total oxygen *plus* carbon dioxide is approximately 21 per cent.

(c) *Internal concentrations of CO<sub>2</sub> at different temperatures.*

The rate of respiration at different temperatures is already known to follow roughly Van 't Hoff's law. Experimental data show that in respect of internal concentrations of CO<sub>2</sub> the same relationship is exemplified.

A large water-melon was fitted with a sampling tube and the internal CO<sub>2</sub> concentration determined at temperatures from 80° F. to 40° F. through progressive stages, in rooms cooled by the air-blast system, sufficient time being given in each chamber to allow the temperature of the flesh to become stabilized. Several estimations were made at each temperature. The fruit was then allowed to regain a temperature of 80° F., estimations again being made at the several intermediate stages. The results are illustrated in Fig. 1. A theoretical Van 't Hoff curve, allowing for a doubling of the rate of chemical change for a rise of 10° C., is shown for comparison.

In a similar series of observations made on large pumpkins considerably lower internal concentrations of carbon dioxide were recorded at all temperatures.

In the apple, Magness (17) has recorded internal concentrations of CO<sub>2</sub> ranging from 6.7 per cent. at 2° C. to 21.4 per cent. at 30° C., and calls attention to the importance of the temperature factor in this aspect of respiration in fruits. Data on the internal carbon dioxide and oxygen concentrations have also been published by Kidd and West (12).

## V. DEVELOPMENTAL PHASE.

(a) *Rates of respiration and transpiration.*

Respiration curves for the apple published by Kidd and West (11) show that in very young fruits the rate of respiration is high and falls off in a regular manner as fruits increase in size, that is, until prior to the rise to the second high (climacteric) value. In other words, with qualifications, the smaller the fruit the higher is the rate of respiration. In a first examination this fact, together with the characteristic shape of respiration curves, might be interpreted as indicating that actual size, involving surface-bulk relationships, is the principal factor determining the amount of CO<sub>2</sub> liberated during respiration; if this were so, the restricting action of the relatively impervious epidermis would acquire additional significance in this particular association. On a further analysis, however, especially where the internal concentrations of carbon dioxide can be precisely determined, the relationship, as will be seen later, is considerably more complicated. Nevertheless, because of the valuable point of view it affords in considering certain aspects

of respiration in fruits, the operation of the size-factor may be briefly restated at this point. Inseparable from all vital phenomena where transmitting surfaces may limit the passage of liquids or gases from one tissue to another, 'it is exhibited in more or less degree in the development of each individual

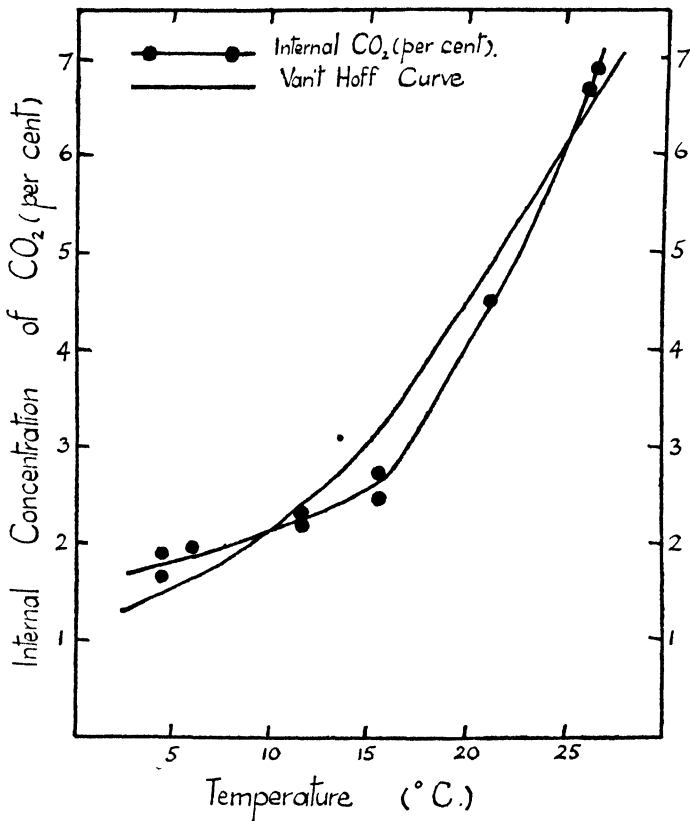


FIG. 1. Internal concentration of carbon dioxide in a large water-melon at different temperatures.

organism and the varying contours of the adult may be held as illustrating in each the balance between the impulse to growth and those modifying conditions under which it has been carried out' (3). In solid objects whatever their shape, provided that on enlargement the same form be maintained, the surface increases as the square, whereas the bulk or volume increases as the cube of the linear dimensions. Accordingly, where progressive changes are taking place in the proportion of surface to bulk in growing cells, tissues, or organs, the far-reaching influence of the size-factor becomes increasingly apparent. As all physiological interchanges are conducted through limiting surfaces, external or internal, the possible action of this factor should not be overlooked in investigating the nature of any collective phenomenon, such,

for example, as respiration. Other things being equal, there is a strong probability that where the surface of interchange is continuous and unbroken, the rate of diffusion of liquids or gases will be directly proportional to the actual area of surface involved. But, according to the Principle of Similarity, if the form be maintained unchanged in a growing cell, tissue, or part, the constantly diminishing proportion of surface to bulk will be directly reflected in decreased rates of interchange. The extent to which the size-factor is operative in determining the rate of respiration in developing fruits obviously merits careful exploration. A consideration of this point of view also directs attention to the distinction which must be drawn between the concept of respiration as measured by the  $\text{CO}_2$  liberated superficially and the total extent of true cellular respiration.

In so far as the size-factor may be operative in other processes involving the superficial liberation of gases, measurements of transpiration have also been carried out for comparison.

*On the nature of surface-volume curves.* If a solid object be progressively enlarged but otherwise unchanged in shape or proportion, and the ratio of surface to volume plotted, curves of the same type shown in Fig. 2 are obtained; whether the curves relate to spheres, cubes, rectangular cuboids or cylinders of different axial proportions, they all have the same characteristic shape. In as much as surface varies as the square of the linear dimensions and bulk as the cube, an approximate theoretical expression of the proportion of surface to bulk is given by  $\frac{2}{3}\sqrt{V}$ , where  $V$  = volume. The

curve for this ratio for different volumes is also illustrated in Fig. 2. In the graphs which follow, this curve has been used as a basis of comparison instead of those which would have been obtained from actual surface and volume measurements of the particular fruits.

*Respiration and transpiration rates.* Curves showing respiration rates for fruits of different sizes are given in Figs. 3, 4, and 5 for the tomato, papaw, and water-melon respectively. Corresponding data are available for other fruits. In general the transpiration rate is approximately ten times that for respiration.

In each instance the similarity of the two curves and their resemblance to the theoretical curve of  $\frac{2}{3}\sqrt{V}$  over a wide size-range is apparent. In other words, it would appear that actual size, i.e. involving surface-bulk relationships, is one factor controlling the rates of transpiration and respiration in the developing fruit.

Notwithstanding the superficial resemblance between respiration, transpiration, and surface-bulk curves, further analysis indicates that this is not so complete an explanation of changing respiration rates as would appear. The rates of liberation of water-vapour in transpiration and of

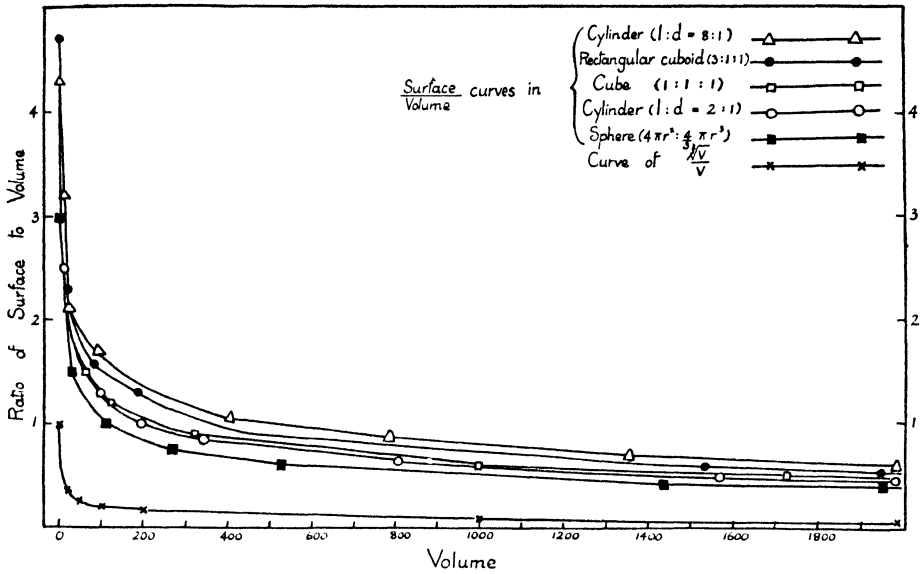


FIG. 2. Curves showing surface-volume relationships over a range in size for various solid objects.

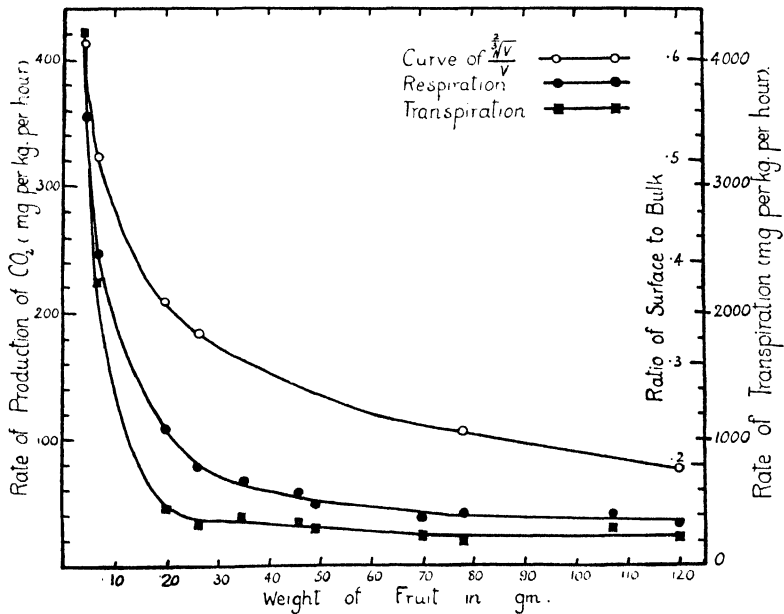


FIG. 3. Respiration and transpiration curves for tomato fruits of different size at 70° F.

CO<sub>2</sub> in respiration depend on the surface-area of the fruit and on the concentration of each at the surface of liberation. With transpiration, where the sub-epidermal tissue is at saturation, the gradient is presumably constant, and the curves depicted may be accepted as an expression of the operation of the size-factor. If we could suppose that the concentration of CO<sub>2</sub> sub-epidermally was also constant during development then the respiration curve could likewise be regarded as exemplifying the operation of the size-factor. From the data now available it is evident that this assumption is not valid. In the following section it will be shown that the internal concentration of carbon dioxide changes throughout development. With CO<sub>2</sub> being continuously produced internally, reduction in the proportion of surface to bulk, or an increase of tissue resistance to the passage of gases, would have little effect on respiration rate, other things being equal; either modification, by bringing about an increase in the internal concentration of CO<sub>2</sub> would automatically be accompanied by the compensating action of a higher gradient and higher rate of liberation. This, however, is not the case.

(b) *Rates of respiration and internal gas concentrations.*

Fig. 6 illustrates throughout the period of active development of the papaw the relationship between internal concentration of carbon dioxide and respiration. This fruit has been found a very convenient object for experimentation.<sup>1</sup>

In Fig. 6 the rate of respiration (mg. per kg. per hour) and the internal concentrations of CO<sub>2</sub> are shown for a series of fruits stripped at the same time from a single tree. The material selected ranged in size from recently fertilized fruits with a weight of 26.68 gm. and a volume of 38 c.c. to full-grown, green fruits with a weight of 1142.9 gm. and volume of 1552 c.c. It will be noted that the two curves run parallel, high values being characteristic of the smaller fruits; prior to the climacteric, approximately steady, lower values are the rule. The curves also show the beginning of the climacteric rise in colouring fruit. *It is noteworthy that an increase in the internal concentration of CO<sub>2</sub> was recorded in fruits which were still quite green externally and which had given no indication of the climacteric rise.*

It may be noted here that in preliminary studies of the internal atmosphere of apples, Dowd (4) observed a tendency for the concentration of

<sup>1</sup> *Carica papaya* a small soft-wooded tree, with an erect stem, is thought to be a native of South America but is widely grown in the tropics. The flowers are usually dioecious, the females, produced in a racemose inflorescence round the crown of the plant being frequently solitary on short stalks. The fruits which somewhat resemble melons are hollow from the earliest stage. They vary greatly in shape, size, thickness of flesh, &c., from tree to tree, but on any one tree the shape is conserved to a marked extent during development. On ripening, the fruits begin to colour on the trees, at which stage they are usually gathered and ripened at tropical temperatures when a full orange yellow colour is developed both on the skin and throughout the flesh. Within, the seeds are borne in five rows along laterally extended peripheral placentae. Most varieties possess a large central cavity.

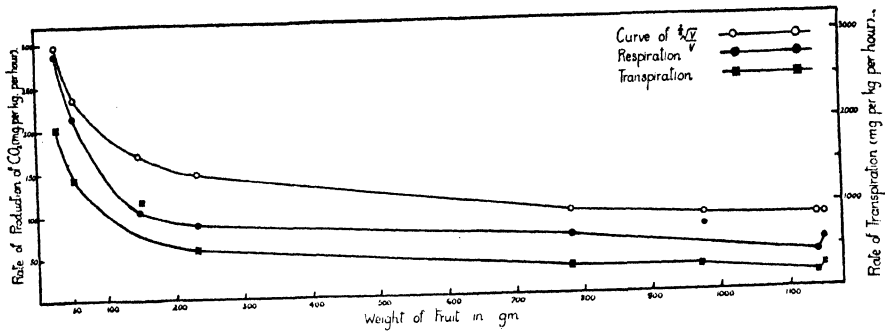


FIG. 4. Respiration and transpiration curves for papaw fruits of different sizes at 80° F.

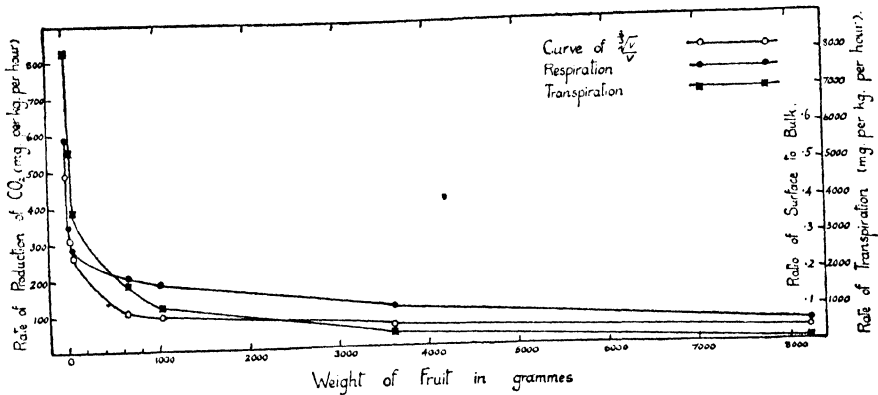


FIG. 5. Respiration and transpiration curves for water-melon fruits of different sizes at 80° F.

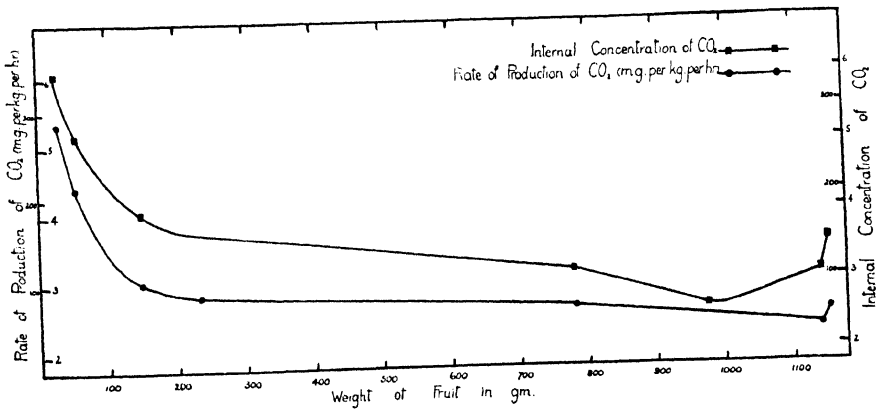


FIG. 6. Internal concentration and rate of production of carbon dioxide in the papaw during development.



CO<sub>2</sub> to decrease during the growing period, while that of O<sub>2</sub> gradually increased.

The high rate of respiration in young, actively-growing tissues has been the subject of much comment. The suggestion has been advanced that this is due to the relatively high proportion of living protoplasm in actively-growing young fruits. In conjunction with the high surface-volume ratio this might have been accepted as the explanation of the high respiration rates observed. The existence of high internal CO<sub>2</sub> concentrations indicates (i) that the high respiration values can be accounted for in terms of a high gradient of gaseous concentration, and (ii) that other factors, not yet elucidated, must be operative to account for the existence of such internal concentrations. In discussing the rapid fall in respiration rate during development, Kidd (16) has pointed out that the standard of reference should be, not unit fresh weight but unit living matter, i.e. omitting cell wall and vacuole. 'Considered thus, the fall during the first period is smaller and may be related to a decrease in enzyme activity.'

In relation to the high respiration rate exhibited by growing fruits, the part played by the developing seeds obviously calls for analytical investigation.

The characteristic shape of the respiration curve for a developing fruit is the result of a complex interaction of factors. Neither the rate of respiration as usually determined, nor the internal concentration of CO<sub>2</sub>, or the two estimations in conjunction, afford an accurate measure of total cellular respiration. Since a high concentration of CO<sub>2</sub> is usually found in the central cavity of the young papaw, it may be inferred that the flesh itself also contains very considerable quantities of this gas, the cavity concentration standing in some relation to that concentration. External liberation of CO<sub>2</sub> over a period of time i.e. respiration as usually measured, thus represents the difference between total cellular respiration and the amount of CO<sub>2</sub> in the cavity and tissues. In view of the high values for CO<sub>2</sub> recorded in the cavities of young fruits, the interesting question arises as to why such relatively large concentration gradients should not result in a still greater liberation of CO<sub>2</sub>. It seems a reasonable inference that the resistance offered by the compact, developing tissue to the passage of gases possesses a further importance in this relation. Summarizing the information now available, it is apparent that a fuller understanding of the course of respiration in developing fruits will depend on (i) a true assessment of the total CO<sub>2</sub> produced (i.e. measuring liberated CO<sub>2</sub>, air-space CO<sub>2</sub>, and CO<sub>2</sub> held in the tissues) and (ii) measurements of the changes in resistance offered to the passage of gases by the epidermis and sub-epidermal tissues during growth of the fruit to adult size.

In view of the complex interaction of the many factors involved in the growth and development of fruits, it is evident that while the operation of

any one factor may be susceptible of exact mathematical exposition throughout this period, the data available are still inadequate. In attempting to ascertain the importance of other factors whose operation may be closely correlated with the foregoing respiration curves, the writers call attention to Gustafson's (6) estimations of percentage dry matter in developing tomatoes; these yield a curve closely akin to those in Figs. 3-6. In the course of tomato respiration studies (7) he attempted to associate intensity of respiration with size of fruit; although his published respiration curves for fruits varying from one to eight weeks old would appear to support this view he came to the conclusion that the operation of the size-factor did not explain the results obtained. As indicated by him, part of the difficulty in establishing the size hypothesis lies in the distinction to be drawn between physiological and chronological age as well as in the interaction of other undetermined factors.

In a recent paper, the same investigator (8) has provided interesting evidence in support of the part played by the size-factor during respiratory processes. In the matter of alcohol production in tomatoes, this was found to be lowest in fruits of small size and to increase progressively in enlarging fruits. Again, rapidly-respiring, orange-coloured fruits of two sizes but of the same physiological age, taken directly from the vines, the larger 10 cm. diameter, on analysis yielded an alcohol content of 0.012 per cent., whereas the smaller, 3.5 cm. in diameter, yielded only 0.0081 per cent. These results presumably are to be referred to proportionately slower access of oxygen to the tissues of the larger fruits under conditions of active metabolism.

## VI. CLIMACTERIC AND SENESCENT PHASES.

So far, the observations submitted have been concerned with immature, growing fruits. With the beginning of ripening, when profound changes begin to take place in the appearance and constitution of skin and flesh, a new phase of respiratory activity is initiated. As, in general, the major growth activities have now ceased, any influence exercised by the size-factor remains constant for each individual. In view of the rapid liberation of  $\text{CO}_2$  during the climacteric phase, it is evident that in order to obtain a more complete knowledge of metabolic activity, careful consideration should be devoted to (i) the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$ , (ii) the changes in resistance to the passage of gases exercised by the tissues of the flesh and skin and (iii) the relation of the tissues of the flesh to the changing concentrations of the several gases involved

### (a) *Preliminary observations on tissue-resistance to gaseous interchange*

The dermal system with its cuticular covering, wax layer where present, lenticels, and stomata—mobile, functional, or modified—shows a wide range of variation. A few examples may be cited in illustration. Thus

the tomato possesses no stomata but shows a thickening of the epidermal cell walls during ripening. The stomata of the papaw apparently possess a certain degree of mobility in the green state but are liable to distortion when shrivelling of the epidermis occurs at late maturity. In the banana,

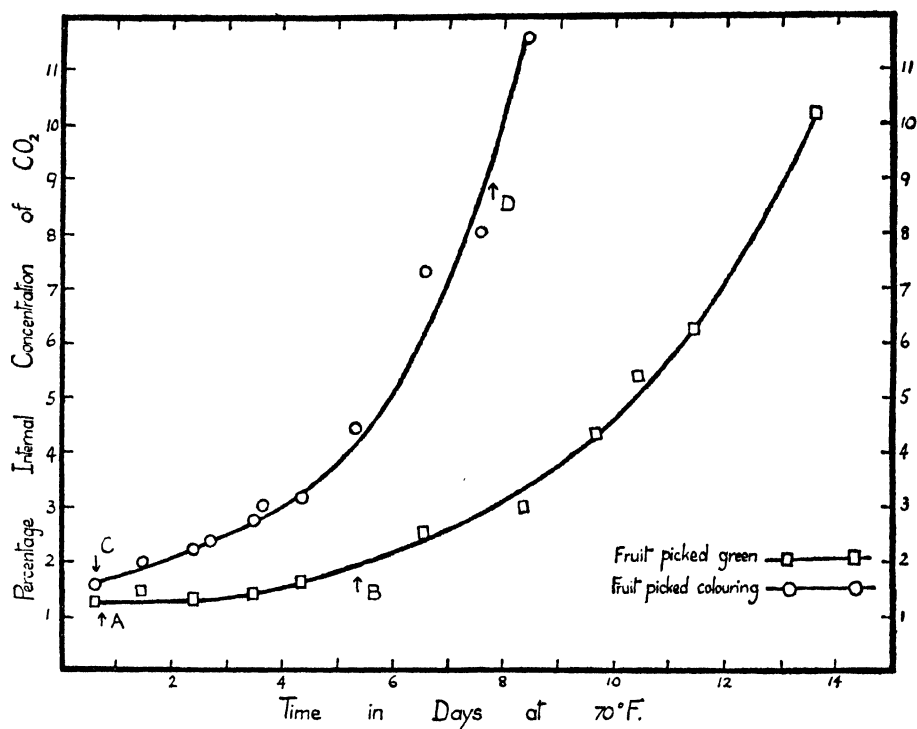


FIG. 7. Internal concentration of carbon dioxide in the papaw during ripening: A, fruit picked green; B, commencement of colouring; C, fruit picked colouring; D, first appearance of anthracnose spots.

when about half-grown, the stomata have a slight aperture but become disorganized during the final growth and ripening processes. That the stomata in bananas and papaws gathered at certain stages of maturity still possess a porosity somewhat higher than that of the epidermal tissue is suggested by the fact that the areas surrounding them are the last to lose their green pigmentation during ripening.

In the apple the stomata as shown by Tetley (19) are of relatively brief existence as functional organs, being subsequently modified to form lenticels. It would appear probable, however, from the experiments of Markley and Sando (18) that a considerable, though variable, proportion of the gaseous interchange takes place through the calyx. This possibility is also envisaged by Kidd and West (12). The closure or non-closure of the stylar passage in individual apples or varieties as a factor in the causation of 'brown heart' may be worthy of consideration.

In order to study alterations in the stomatal aperture porometric methods as used in the study of leaf stomata were attempted, but it was found that with unripe papaws and bananas a difference of pressure considerably in excess of that used with leaves was necessary, a circumstance liable to produce undesirable tissue strains. On sectioning such tissues, this result was seen to be explicable by the smallness of the sub-stomatal air-chambers and the paucity of connecting intercellular air-spaces.

The porosity of the path of gaseous interchange in the papaw was next examined by taking, with a cork-borer, plugs of tissue extending from epidermis to internal air-space, applying suction and observing the rate of flow of air as with the porometer. Only slight suction was required in fruits with green epidermis and white or pale yellow flesh, but in yellow, watery-fleshed papaws subjected to similar conditions no appreciable air-flow could be demonstrated; under the higher pressures used to induce gas movement in such older fruits the tissues became compacted.

Similar papaw plugs were also utilized in studying the permeability of fruit tissue to gases: the plug was enclosed in a glass tube, a high concentration of  $\text{CO}_2$  applied on the inner side and a stream of air drawn over the epidermis; the  $\text{CO}_2$  passing through the plug was then absorbed in Pettenkofer tubes. Similar plugs without supplied  $\text{CO}_2$  were used as controls. The preliminary results obtained indicate that the permeability of plugs from immature fruits is greater than that of ripe fruits. Other measurements of the passage outwards of  $\text{CO}_2$  and inwards of  $\text{O}_2$  made with whole papaw fruits, using the technique outlined in Section IV, demonstrate the same decrease of permeability with ripening. The relative resistance offered by the epidermis and other tissues will be the subject of further experiments.

(b) *Rates of respiration and internal concentrations of  $\text{CO}_2$ : preliminary observations.*

In full-grown, green papaws, which have given no indication of the initiation of the climacteric phase, as judged by the usually accepted criteria, viz. increased rate of 'respiration' and commencement of skin coloration, it has been ascertained as stated, that the internal concentration of  $\text{CO}_2$  has already begun to increase. This increase continues steadily during the colouring, softening, and final maturation of the fruit. Fig. 7 illustrates some preliminary observations on the internal concentrations of  $\text{CO}_2$  in a standard type of papaw (i) in a fruit picked full-grown green and (ii) in a riper fruit, picked when the first traces of skin yellowing had appeared. Fig. 8 also shows respiration curves and internal  $\text{CO}_2$  concentrations for several fruits. From such observations it is evident that the rise to the peak value during the climacteric (i.e. as measured in studies of 'external'

respiration) is directly related to increasing internal  $\text{CO}_2$  concentration. But whereas in the measurement of 'external' respiration there is a falling off in the rate of  $\text{CO}_2$  liberation after the peak has been passed, no corresponding decrease in the internal concentration of  $\text{CO}_2$  is apparent. In

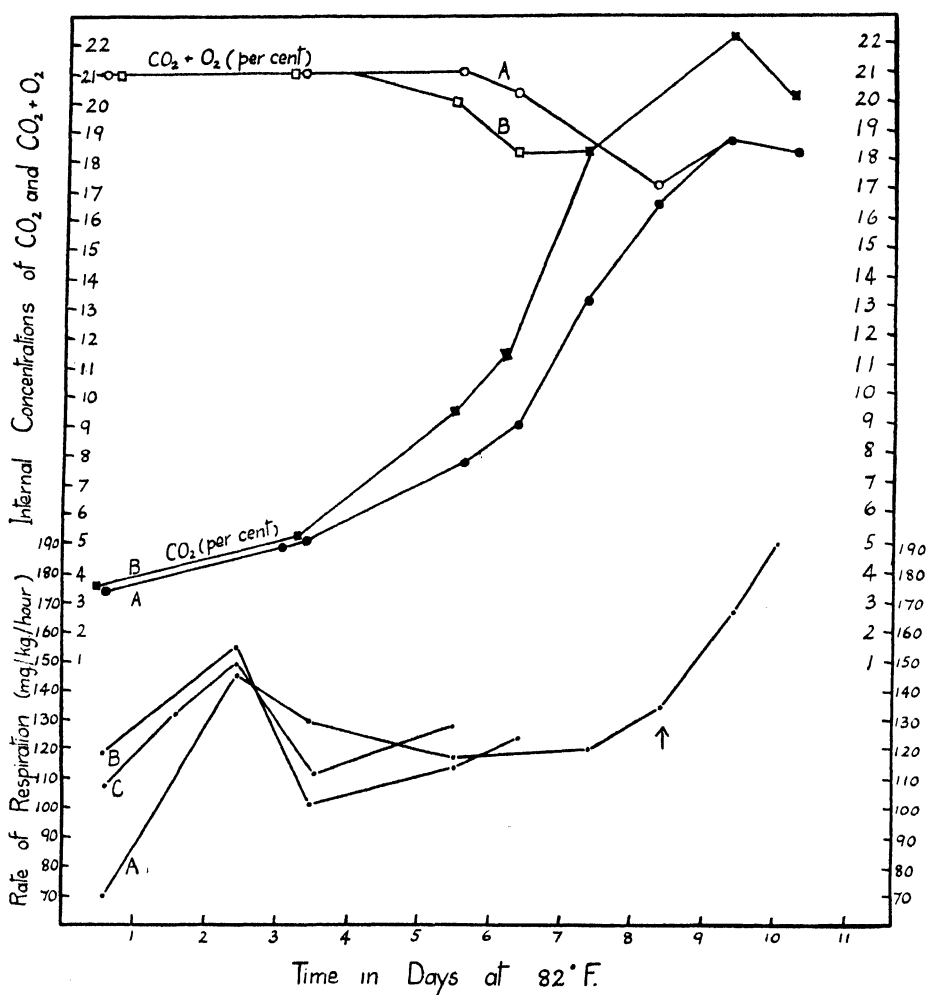


FIG. 8. Rate of respiration and internal concentrations of  $\text{CO}_2$  and of  $\text{CO}_2 + \text{O}_2$  in the papaw during ripening. A, B, and C are three fruits. The lowermost curves show the climacteric rise during ripening; the arrow-head indicates the onset of anthracnose spots. Internal concentrations of  $\text{CO}_2$  are shown for fruits A and B only, those for C occupying an intermediate position.

other words, whereas the characteristic respiration curve for a ripening fruit suggests a marked decline in the rate of metabolism after the peak value has been passed, it is evident that this view requires further examination in the light of the information now available on the internal gas concentrations of fruits. The fact that the internal  $\text{CO}_2$  concentration

continues to rise while the external liberation of  $\text{CO}_2$  is falling off indicates that those processes involved in the colouring and softening of fruits are likewise responsible for an increased resistance to the outward diffusion of  $\text{CO}_2$  and inward diffusion of  $\text{O}_2$ .

Using Bramley's Seedling apples, Kidd and West (15) have shown that the difference in concentration (both for  $\text{O}_2$  and  $\text{CO}_2$ ) between the external and internal atmospheres, when plotted against time, gives a curve similar to that for respiratory activity. From these internal gas concentrations and respiration values, a third value can be deduced which is a rough measure of the porosity of the apple: the values for porosity were approximately constant up to the climacteric rise but gradually decreased after that point.

(c) *Internal concentrations of  $\text{O}_2$  and  $\text{CO}_2$ .*

Following the preliminary experiments outlined above, several series of observations on the internal oxygen and carbon-dioxide concentrations throughout the course of maturation, senescence, and final decay in the papaw were made and may now be considered in detail. The results for five fruits are shown in graphic form in Figs. 9-13. As these represent the first observations of this kind to be published (as far as the writers are aware) it has been thought fit that the data should be presented in some detail.

In each instance, the fruit was fitted with a glass tube, vaselined washer, and clipped rubber tube. Fruits from three trees were used, a green and a colouring fruit from two of the trees being stored at  $80^\circ\text{F}$ . and a green fruit from the third being stored at  $70^\circ\text{F}$ . Samples were drawn out at intervals and the concentrations of  $\text{CO}_2$  and  $\text{O}_2$  determined. But whereas in the measurement of 'external' respiration the experiment is usually concluded when superficial wastage begins to appear—with concomitant sharp increase in the respiration rate—in these studies it was found possible to continue, with advantage, the recording of internal concentrations. Accordingly fruits were not discarded until they had reached an advanced state of rotting. The value of such records is apparent when it is recalled that, in the normal course of fruit-fall and decay in a state of nature, the seeds lining the internal cavity are subject to comparable vicissitudes.

*Tree A. Furrowed Ovoid Fruit, with Star-shaped, Well-seeded Cavity.*

*Fruit 1* (Fig. 9). Picked green with first trace of yellow in furrows; volume 979.5 cc.; internal volume 90 cc.; weight 799.5 gm. (Ripened at  $80^\circ\text{F}$ .)

Concentration of  $\text{CO}_2$  on picking: 3.79 per cent.

"	$\text{O}_2$ "	: 17.29	"
"	$\text{CO}_2 + \text{O}_2$ "	: 21.08	"

Time to reach climacteric peak value : 2 days.

Time to become full yellow : 3-4 days.

$O_2 + CO_2$  concentration maintained at 21 per cent. (approx.) : 4 days.

( $CO_2 = 5.96$  per cent. ;  $O_2 = 14.87$  per cent.)

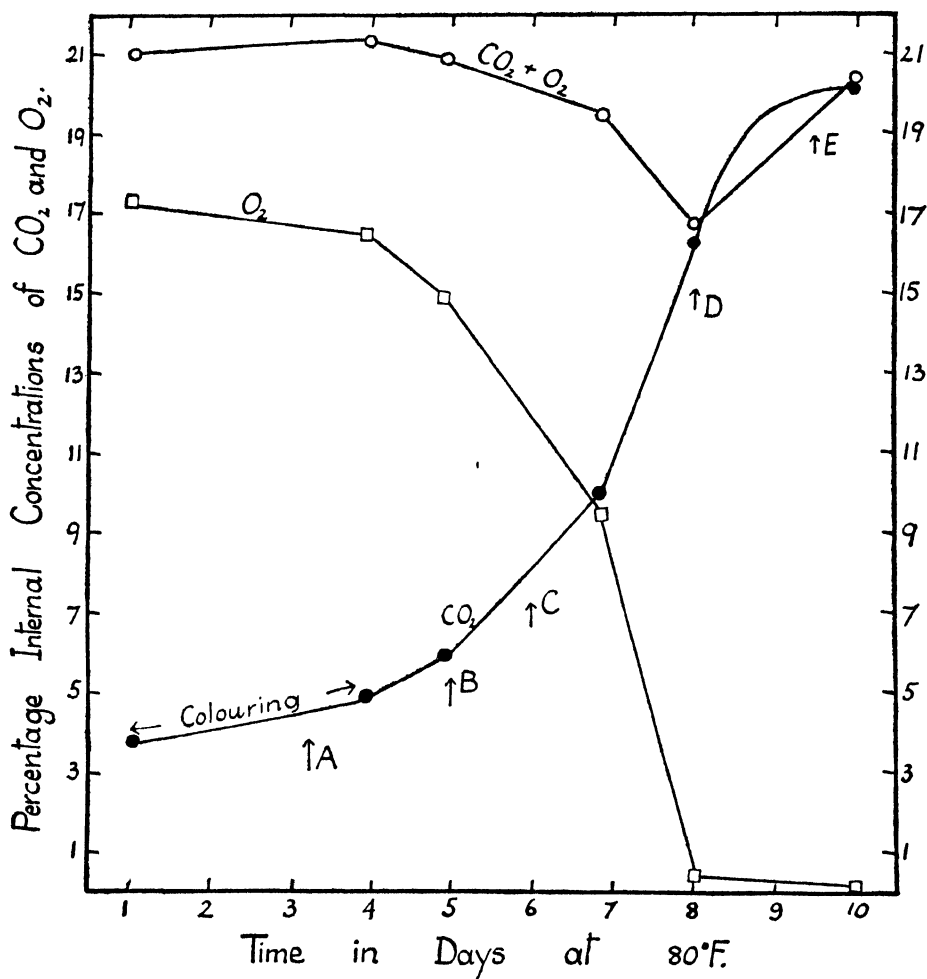


FIG. 9. Internal concentration of carbon dioxide, oxygen, and carbon dioxide *plus* oxygen during the ripening of the papaw at 80° F. A, relative position of climacteric peak value; B, fruit fully coloured; C, first appearance of anthracnose spots; D, severe wastage; E, evidence of outward leakage of  $CO_2$  from fruit cavity due to severe rotting.

Appearance of anthracnose spots : 5 days.

( $CO_2 = 7.90$  per cent. ;  $O_2 = 14.87$  per cent.)

Concentration of gases on 7th day :  $CO_2 = 16.25$  per cent. ;  $O_2 = 0.42$  per cent.

( $CO_2 + O_2 = 16.67$  per cent.)

Severe wastage with abundant secondary fungi present : after 7th day.

Gas concentration on 9th day, with internal fungal activity.

( $CO_2 = 20.19$  per cent. ;  $O_2 = 0.10$  per cent.)

*Fruit 2* (Fig. 10). Full orange-yellow colour on picking, i.e. climacteric peak value already past; volume 1655 cc.; internal volume 120 cc.; weight 1280 gm. (Ripened at 80° F.)

Concentration of  $\text{CO}_2$  on picking: 5.37 per cent.

"  $\text{O}_2$  " : 16.13 "

"  $\text{CO}_2 + \text{O}_2$  " : 21.50 "

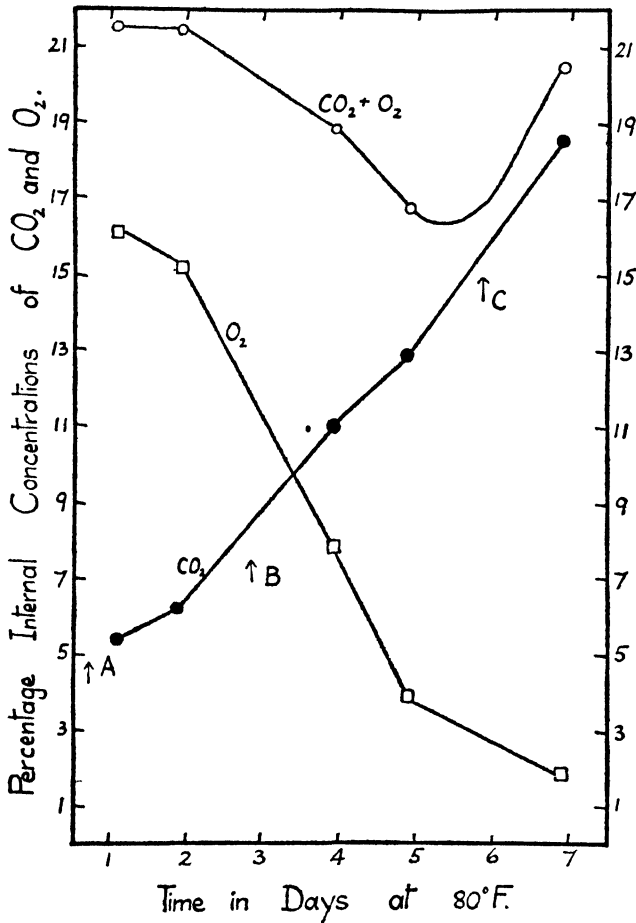


FIG. 10. Internal concentration of carbon dioxide, oxygen, and carbon dioxide *plus* oxygen during the ripening of the papaw at 80° F. A, fruit yellow on picking; B, appearance of anthracnose spots; C, onset of severe wastage.

$\text{CO}_2 + \text{O}_2$  maintained at 21 per cent. (approx.): 1 day.

Appearance of anthracnose spots: 2 days.

( $\text{CO}_2 = 8.40$  per cent.;  $\text{O}_2 = 11.90$  per cent.)

Concentration of gases on 4th day:  $\text{CO}_2 = 12.87$  per cent.;  $\text{O}_2 = 3.95$  per cent.

( $\text{CO}_2 + \text{O}_2 = 16.82$  per cent.)

Onset of severe wastage: 5th day.

Gas concentration on 7th day (severe wastage):  $\text{CO}_2 = 18.56$  per cent.

( $\text{O}_2 = 1.91$  per cent.;  $\text{CO}_2 + \text{O}_2 = 20.47$  per cent.)



*Tree B. Furrowed Sub-spherical Fruit of Large Size, with Well-seeded Cavity.*

*Fruit 1* (Fig. 11). Picked full-grown, green; volume 2662 cc.; internal volume 235 cc; weight 1979 gm. (Ripened at 80° F.)

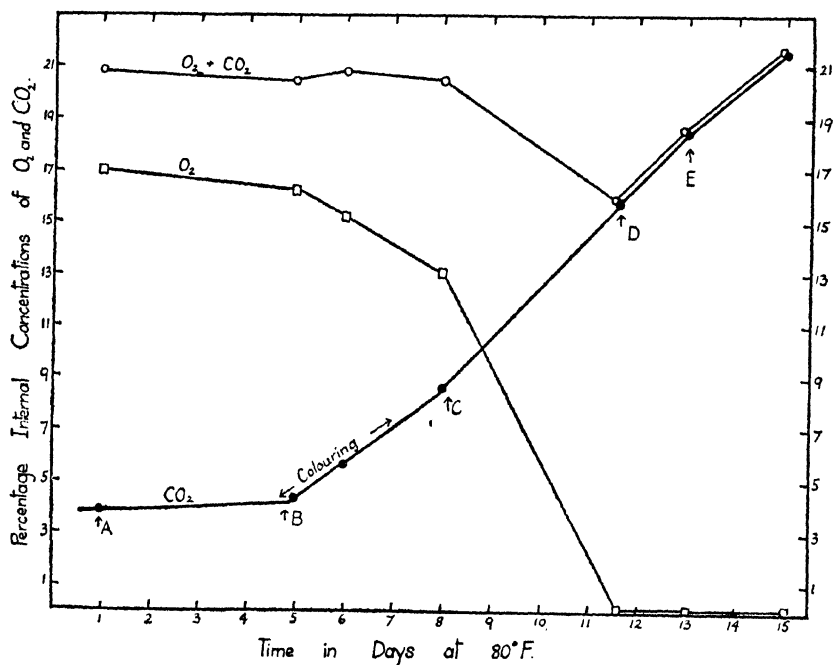


FIG. 11. Internal concentration of carbon dioxide, oxygen, and carbon dioxide *plus* oxygen during the ripening of the papaw at 80° F. A, fruit picked green; B, commencement of colouring; C, fruit fully coloured and first appearance of anthracnose spots; D, severe wastage; E, rapid increase in internal CO<sub>2</sub> concentration due to yeasts, &c. in fruit cavity.

Concentration of CO<sub>2</sub> on picking : 3.89 per cent.

" O<sub>2</sub> " 17.03 "

" CO<sub>2</sub> + O<sub>2</sub> " 20.92 "

Commencement of skin colouring : 4th day.

Fruit fully coloured : 6th day.

(CO<sub>2</sub> = 7.10 per cent. ; O<sub>2</sub> = 14.20 per cent.)

Appearance of anthracnose spots : 7th day.

(CO<sub>2</sub> = 8.49 per cent. ; O<sub>2</sub> = 12.07 per cent.)

Concentration of gases on 11th day : CO<sub>2</sub> = 15.70 per cent. ; O<sub>2</sub> = 0.01 per cent.

(CO<sub>2</sub> + O<sub>2</sub> = 15.71 per cent.)

(Fruit badly diseased with secondary fungi present.)

Concentration of gases on 15th day (internal fungal activity) : CO<sub>2</sub> = 21.56 per cent. ;

O<sub>2</sub> = 0.03 per cent.

(CO<sub>2</sub> + O<sub>2</sub> = 21.59 per cent.)

*Fruit 2* (Fig. 12). Fruit with traces of colour on picking; volume 2448 cc.; internal volume 220 cc.; weight 1823 gm. (Ripened at 80° F.)

Concentration of  $\text{CO}_2$  on picking : 4.03 per cent.

"  $\text{O}_2$  " 16.85 "

"  $\text{CO}_2 + \text{O}_2$  " 20.88 "

Fruit fully coloured : second day.

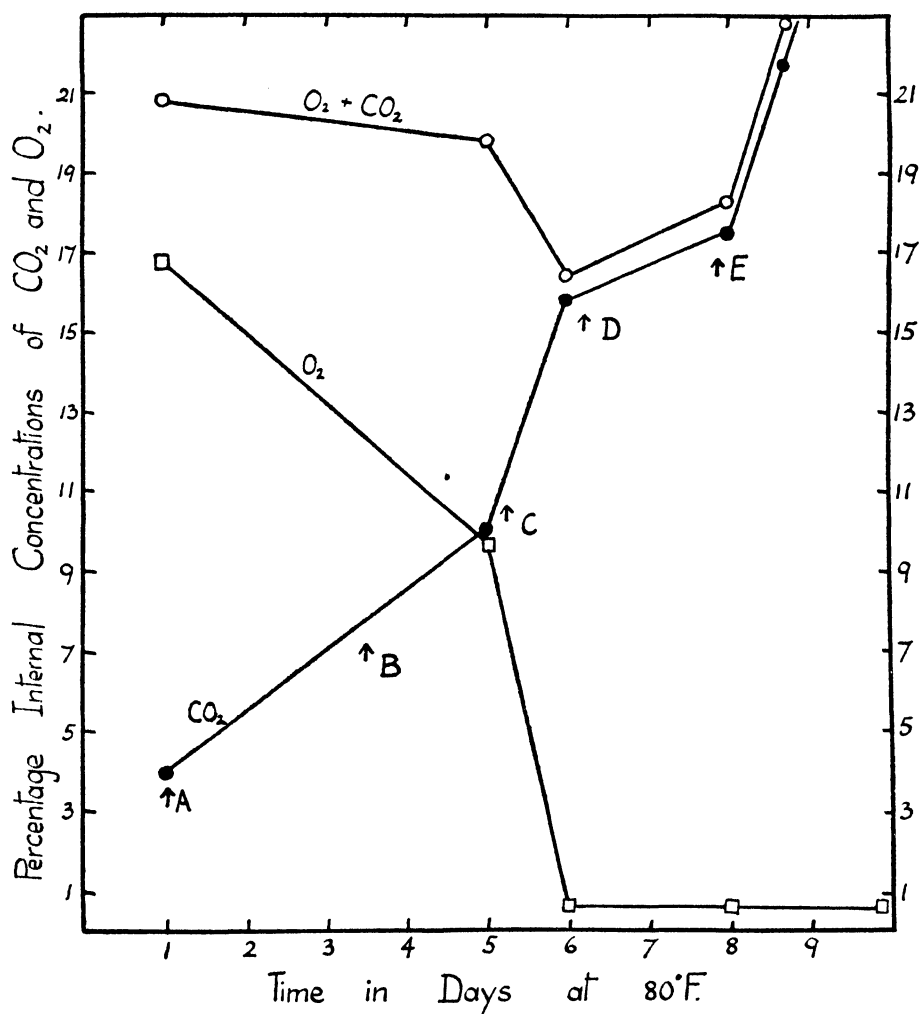


FIG. 12. Internal concentration of carbon dioxide, oxygen, and carbon dioxide *plus* oxygen during the ripening of the papaw at 80°F. A, traces of colour on picking; B, fruit fully coloured; C, appearance of anthracnose spots; D, severe wastage; E, rapid increase in internal  $\text{CO}_2$  concentration due to yeasts, &c. in cavity.

Appearance of anthracnose: 3rd day.

( $\text{CO}_2$  = 7.80 per cent.;  $\text{O}_2$  = 12.50 per cent.) ( $\text{CO}_2 + \text{O}_2$  = 20.30 per cent.)

Wastage severe: 6th day.

( $\text{CO}_2$  = 15.83 per cent.;  $\text{O}_2$  = 0.65 per cent.) ( $\text{CO}_2 + \text{O}_2$  = 16.48 per cent.)

Gas concentration on 12th day (very severe wastage, with yeasts and other fungi inside):  $\text{CO}_2$  = 61.45 per cent.;  $\text{O}_2$  = 0.69 per cent.

*Tree C. Large, Furrowed, Sub-spherical Fruit with Large Cavity.*

*Fruit 1* (Fig. 13). Fruit picked full-grown green; volume 4178 cc.; internal volume 1410 cc.; weight 2473 gm. (Ripened at 70° F.)

Concentration of  $\text{CO}_2$  on picking: 1.57 per cent.

"  $\text{O}_2$  " 19.30 "

"  $\text{CO}_2 + \text{O}_2$  " 20.87 "

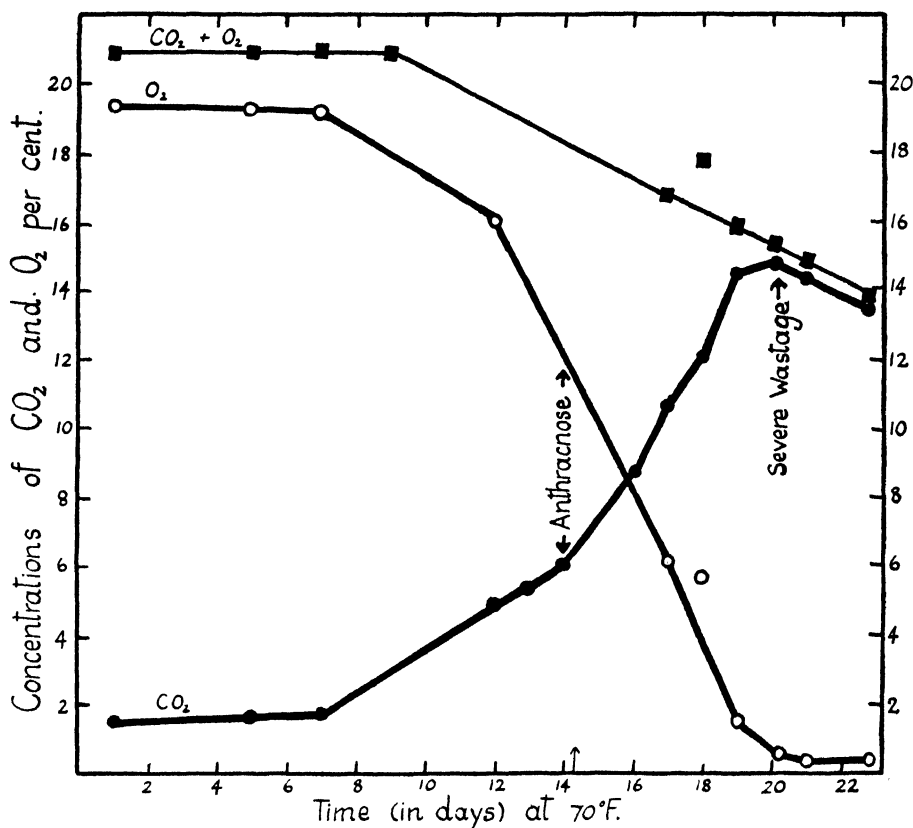


FIG. 13. Internal concentration of carbon dioxide, oxygen and carbon dioxide plus oxygen during the ripening of the papaw at 70° F.

Commencement of yellowing: 6th day.

Spread of yellowing: 6th to 10th day.

Gas concentration on 11th day:  $\text{CO}_2$  = 4.87 per cent;  $\text{O}_2$  = 16.00 per cent.

( $\text{CO}_2 + \text{O}_2$  = 20.87 per cent.)

Appearance of anthracnose spots: 14th day.

( $\text{CO}_2$  = 6.06 per cent.;  $\text{O}_2$  = 12.00 per cent.) ( $\text{CO}_2 + \text{O}_2$  = 18.06 per cent.)

Severe external wastage, but no internal wastage: 21st day.

( $\text{CO}_2$  = 14.82 per cent.;  $\text{O}_2$  = 0.53 per cent.) ( $\text{CO}_2 + \text{O}_2$  = 15.35 per cent.)

(d) *Discussion of internal gas concentrations.*

With minor variations, the ripening process is accompanied by an increase in the internal concentration of  $\text{CO}_2$  and a corresponding decrease

in  $O_2$ , the sum of the percentages of the two gases being maintained in the initial stages at approximately 21 per cent. As far as measurements of internal  $CO_2$  concentrations are concerned, there are no modifications in the curves corresponding to the climacteric peak value shown by 'respiration' curves. *It would appear that the occurrence of a peak value (in the papaw at least) is partly attributable to a change in the resistance to gaseous interchange on the part of the tissues during ripening.*

It is characteristic of the papaw that when a certain stage of ripeness is reached the fruit becomes spotted with the anthracnose fungus, *Colletotrichum gloeosporioides*. Mycological experience shows that this fungus is established as a field infection at an early stage but remains dormant until a certain degree of maturity is reached. The spots are circular, slightly sunken, and for the most part superficial, the chief damage being a destruction of epidermal and sub-epidermal tissues. Later, these spots serve as a means of ingress for rapidly-growing secondary fungi or wound parasites; thereafter wastage goes on apace so that the flesh is rotted through and the cavity finally becomes infected.

In 'external' respiration studies, the first appearance of anthracnose spots is accompanied by a marked rise in the 'rate of respiration'; with the extension of the spots this continues. A common interpretation of this phenomenon—the end-point of many respiration studies—is that the carbon dioxide is due, not so much to metabolic processes in the fruit, but rather to the active metabolism of the fungi which have become established. Some modification of this view is now necessary. In the case in point, the progress of the fungus through the flesh is slow and the total volume of tissue exploited correspondingly small. The major feature of the disease is, in fact, a destruction of superficial tissue. But as active metabolism still continues in the fruit flesh, and as the internal concentration of carbon dioxide continues to rise steadily, and, by inference, the  $CO_2$  content of tissues, a gradient of gaseous concentration of considerable steepness is accordingly created. Meanwhile the epidermal and sub-epidermal areas destroyed by fungal activity afford a passage by which increased leakage from the tissues can take place. Thus the rise in 'respiration rate' coincident with the onset of wastage may now be re-assessed as being chiefly referable to leakage through the partly-destroyed epidermis under conditions of high internal carbon-dioxide concentrations, the part played by fungal metabolism in the initial stages being of minor importance. Various aspects of respiration after wounding are likewise susceptible of similar interpretation.

The phase in ripening characterized by the appearance of anthracnose spots is accompanied by notable changes in the relative gas concentrations. From this time onwards the concentration of oxygen falls rapidly while that of carbon dioxide increases. The sum of the two percentage

concentrations, however, now falls to less than 21 per cent., values in the neighbourhood of 16 per cent. being common when all the oxygen has practically disappeared. Hence the diminishing  $O_2$  values must represent replacements by  $CO_2$  and  $N_2$ . The explanation of these changes would appear to be as follows: with increased resistance of post-climacteric, ripening tissue to the movement of gases, oxygen, though still slowly diffusing inwards (together with  $N_2$  in atmospheric proportions) no longer gains access at a sufficient rate to keep pace with metabolic requirements; accordingly, as metabolism is evidently still active, the cavity oxygen is utilized and replaced by  $CO_2$  and  $N_2$ . The causes underlying such changes in tissue resistance suggest themselves as an obvious sequel to the present research.

It will be seen that the changes described above involve a diminution in the percentage of internal oxygen, a zero value being finally obtained. At this stage, when fungal activity has usually become extensive, the concentration of carbon dioxide may actually diminish through increased leakage: this is of short duration, however, the access of rapidly-growing organisms to the central cavity soon resulting in a sharp rise. Where yeasts gain access, the carbon dioxide value may become very high indeed, concentrations of 60 per cent. and over having been recorded. At this stage no further estimation of the fruit's respiratory activity is possible by this method.

In these studies of ripening in the papaw it has been seen that in the later stages of maturation the amount of oxygen in the cavity and, by inference, in the tissues is very small. In other words, the later phases of senescence are characterized by a marked transition from aerobic to anaerobic respiration. The relation which the accompanying conditions of internal gas concentration bear to the rotting of tissues by fungal organisms raises questions of considerable interest. Thus the destructive activity of the anthracnose organism, a definite pathogen, has been found to be delayed until the internal oxygen concentration has fallen to 12 per cent. or less. On the other hand, the considerable incidence of more purely saprophytic fungi is not apparent until the tissues are virtually under anaerobic conditions. To what extent the several aspects of decreased resistance to fungal attack are referable to reduced oxygen content or to the depressant action of high concentrations of carbon dioxide is a line of investigation well worth further consideration.

(e) *Respiration of pods of pigeon pea.*

For comparison with the papaw an extreme example was sought in the small pods of the pigeon pea (*Cajanus indicus*). The pods are about 2 inches long, and constricted between the seeds, generally four in number. With maturation the fruits become indurated and coloured, and the seeds

very hard. As the pods can be slipped easily into the standard Haldane gas-sampling bottle, a simple, accurate method of estimating production of  $\text{CO}_2$  was thus afforded.

The following procedure was adopted: Pods representing five stages of maturity were selected, weighed, and the volume determined. Each was then placed in a sampling bottle of known volume and the amount of  $\text{CO}_2$  liberated estimated at the end of  $2\frac{1}{2}$  hours. Using a sharp scalpel the pods were then opened along one suture, and the seeds carefully removed. The separate weights of pods and seeds were then obtained; the requisite measurements of volumes of seeds and empty pods were obtained at the completion of the experiment. Seeds and pods were placed in separate sampling bottles, and the amount of  $\text{CO}_2$  liberated measured after  $2\frac{1}{2}$  hours. The experiment was conducted at  $70^\circ \text{F}$ .

The relevant data are set out in the following Tables.

TABLE I.

Sample No.	Maturity.	Weight of entire pod (gm.).	Weight of seeds (gm.).	Weight of pod without seeds (gm.).
1	Not full grown	1.258	0.770	0.488
2	Almost full grown	1.440	0.960	0.480
3	Full grown, pod colouring	1.539	1.020	0.519
4	Almost mature; seeds hard	0.703	0.520	0.183
5	Mature; seeds hard	0.740	0.540	0.200

TABLE II.

*'Rates of Respiration' (Mg. of  $\text{CO}_2$  per Kg. per Hour).*

Sample No.	Entire pod.	Seeds alone.	Opened pods without seeds.
1	593.7	929.0	892.1
2	386.8	619.3	709.2
3	362.4	458.5	570.3
4	0	178.2	407.4
5	0	111.0	504.0

TABLE III.

*Total Amounts of  $\text{CO}_2$  Liberated in 'Respiration' (in Mg. per Hour).*

Sample No.	Unopened pod.	Seeds alone.	Opened pod alone.	Seeds plus opened pod.
1	746.9	638.4	435.3	1073.7
2	557.1	594.3	340.3	934.6
3	557.7	467.7	296.0	763.7
4	0	92.7	74.6	167.3
5	0	59.9	100.8	160.7

In the hard, dry, indurated pods (Samples 4 & 5) no  $\text{CO}_2$  could be detected at the end of  $2\frac{1}{2}$  hours—an observation confirmed when the experiment was repeated.

Even in a diminutive fruit such as the pea pod, whose shape affords a relatively high surface for gaseous interchange, it is evident that the amount of  $\text{CO}_2$  measured in 'external' respiration studies does not give a true picture of the actual production of  $\text{CO}_2$ . Kertesz (10) has demonstrated internal concentrations of  $\text{CO}_2$  in pea pods amounting to 1.6 per cent. The pod tissues, especially in the more mature fruits, are again seen to offer considerable resistance to the passage of gases.

(f) *The initiation of ripening.*

A brief commentary may be offered on the processes involved in the initiation of ripening in fruits—a phenomenon for which no satisfactory account, so far as the writers are aware, has hitherto been forthcoming. In the papaw the central cavity is lined with seeds disposed along five broad placentae. In young fruits the seeds are relatively small and white in colour. In progressively older fruits the seeds, still white, undergo a considerable increase in size. At the point where the seeds have more or less attained full size, both the respiration curve and that for internal carbon-dioxide concentration begin to lie approximately parallel to the horizontal axis, indicating a declining but relatively steady rate of carbon-dioxide production. At this stage the fruit is still increasing in weight. The fully-formed seeds now begin to develop a brownish colour, and before the climacteric is reached have become quite dark. This represents the completion of seed development, the darkening colour being the visible evidence of the final maturation and induration of the seed coat, Fig. 14. At this stage the fruit flesh is quite white and the skin dark green in colour in marked contrast to the orange-yellow colour of the skin and flesh of the fully ripened fruit. The completion of seed formation as a phase in regional development apparently now acts as the directive stimulus in the initiation of ripening processes. In favourable material, such as the papaw, it can readily be demonstrated that the very first trace of ripening colour occurs in the fleshy stalks on which the individual seeds are borne; ripening colour is next seen in the placentae, and the spread of colour then follows the main vascular tracts, which occupy the tissue between the placentae internally and the furrows externally. That the first evidence of external colour is to be seen in the furrows is in keeping with the scheme outlined. In orderly sequence ripening finally follows the secondary vascular strands, till a uniform, orange-yellow colour is present throughout the flesh. The close relation that exists between the onset of ripening and changes in the internal concentration of carbon dioxide is shown in Fig. 14.

Allowing for differences in the construction of the fruit, an essentially

similar ripening scheme can be demonstrated in the tomato. In full-grown, green fruits, where the seeds have acquired mature seed coats, the first trace of pink or red colour develops in the placenta immediately adjacent to the seed clusters. Colour changes then follow the path of the major vascular

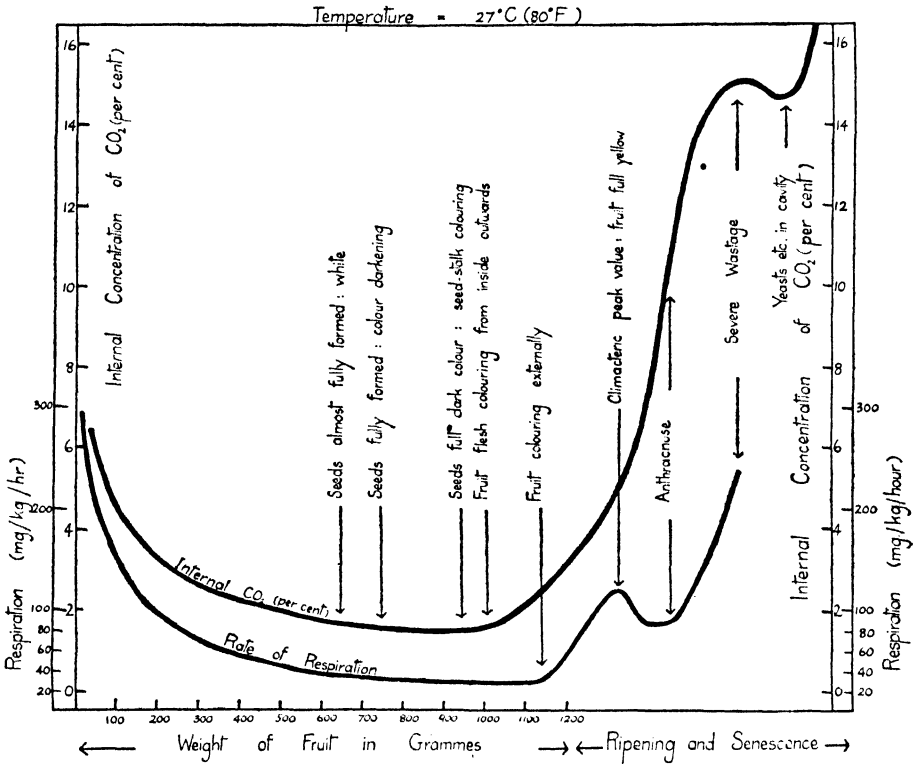


FIG. 14. Rate of production and internal concentration of carbon dioxide throughout the development, ripening, and senescence of the papaw at 80° F. (semi-diagrammatic).

strands. In contrast to the papaw, these lie in the tomato between the placenta and the external ridges of the fruit. Hence in the tomato, the fact that red colour first appears on the ridges may be taken as confirmation of the scheme outlined. Other fruits examined confirm the observation that ripening proceeds from the seeds or stone outwards to the skin.

In the previous section it was shown for pods of *C. indicus* that fully-grown, hard seeds have a very low rate of respiration; this is in keeping with current knowledge of the respiration of other dry mature seeds. It is to be noted that final maturity in seeds, with concomitant low respiration rate, marks the point in time at which the internal  $O_2$  concentration of fruits such as the papaw reaches its maximum point. From the facts (i) that in young, developing fruits the sum of the concentrations of  $O_2$  and



CO<sub>2</sub> is 21 per cent., and (ii) that CO<sub>2</sub> shows a declining concentration during development, it is evident that the curve of internal oxygen concentration shows a peak value corresponding in time with (a) final maturation of seeds, and (b) the initiation of the rise in internal CO<sub>2</sub> concentration closely

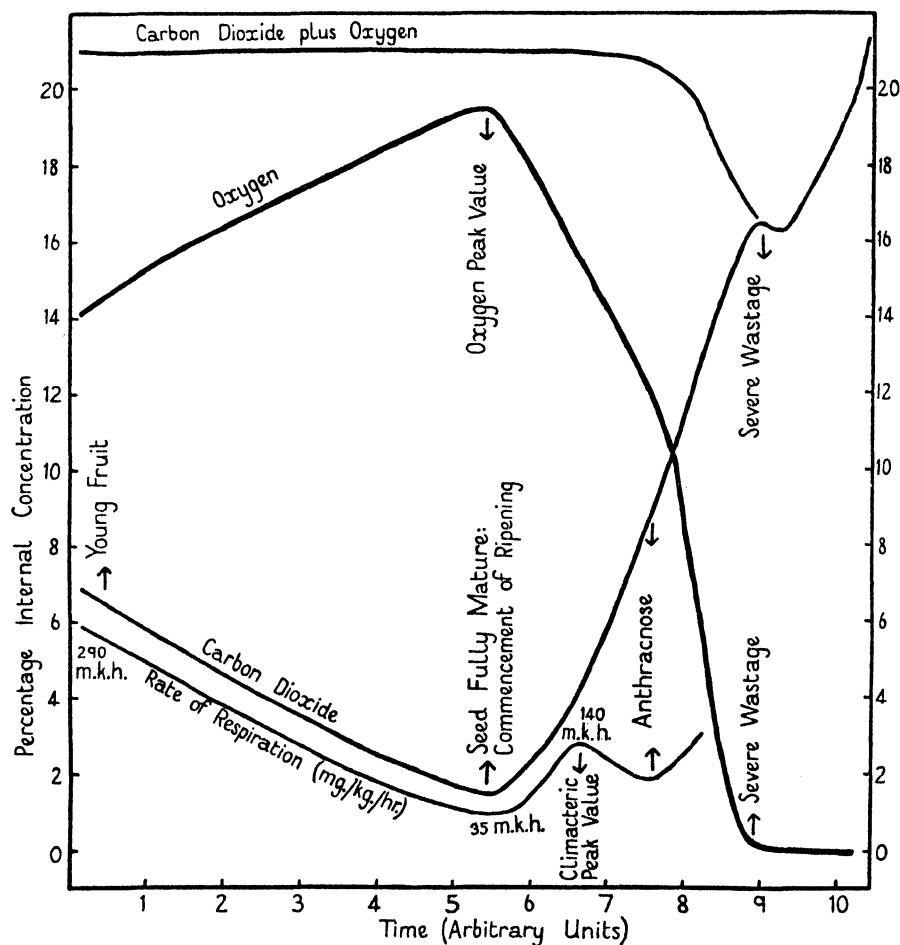


FIG. 15. Diagrammatic representation of internal gas concentrations and of respiration rate during the development, ripening, and senescence of the papaw.

followed by the climacteric. In fact, in the form of a graph, the O<sub>2</sub> concentration is a mirror image of that of CO<sub>2</sub> until the sum of the concentrations falls below 21 per cent., Fig. 15.

In relation to the foregoing observations, a fund of interesting commentary obviously falls to be made concerning the initiation of ripening processes in those fruits such as the banana and bread-fruit which are seedless. This, however, is beyond the scope of the present paper.

## VII. GENERAL DISCUSSION.

The outstanding feature of respiration curves for ripening fruits is, first of all, a rise to the peak value, more or less coincident with the onset of colouring, followed by a decline. In earlier researches, in which it has been assumed that the respiration curve affords an approximate picture of metabolic drift, the inference has been that the maximum rate of metabolic activity coincides with the peak value, this being followed by a gradual decrease with the advance of senescence. With perhaps a few exceptions, however, difficulty has been experienced in indicating any exact correlation between biochemical changes in the flesh of the fruit and such changing respiration rates.

Blackman and Parija (2) consider that the rise in the rate of  $\text{CO}_2$  production is due to a protoplasmic factor, in particular to changes in 'hydrolysis resistance' permitting of an increased rate of production of the effective respiratory substrate. Kidd and West (13) have called attention to 'the lack of evidence that the rise in respiratory activity at the beginning of the senescent phase in the life-history of the apple is associated with any corresponding change in the concentration of any of the estimated constituents of the cells, and have tentatively put forward the hypothesis that the rise is due to "a change of state" in the colloidal matrix of the protoplasm.' The types of change envisaged include those leading to (i) a greater amount of effective enzymes and (ii) a greater effective concentration of the substrate, 'either by elution or by increase in permeability of the surface of the protoplasm bordering on the cell vacuole'. In indicating the significance of changes in protoplasmic state, Kidd and West refer to the fact that the temperature factor, whose operation is very marked where respiration rate is concerned, is likewise of fundamental importance in 'phenomena associated with changes in colloidal state, such as the denaturation of proteins, the inactivation of enzymes, and the life-duration of seeds. It is probable, therefore, that the acceleration and deceleration of respiratory activity observed during senescence is principally due to changes in colloidal state of the protoplasm'.

In the tomato Gustafson has suggested that the climacteric rise is due to a lowering of the H-ion concentration of the cell-sap (7).

For the apple, Kidd (16) indicates that, at the climacteric, fructose—the substrate of respiration—begins to disappear for the first time, while the sucrose content rises; the concentration of cane sugar and rate of respiration later reach a maximum simultaneously at the climacteric peak and thereafter decline together. The rate of respiration during this phase is determined by the concentration of active fructose in the vacuole, the seat of the reaction throughout being the interface between the cytoplasm and the vacuole. Prior to the climacteric it is thought that normal

fructose and cane sugar do not penetrate from the cytoplasm into the vacuole; at the beginning of the climacteric, on the other hand, it is suggested that with the attainment of a critical hydrogen-ion concentration in the vacuole, a change in permeability of the cytoplasm to fructose takes place, whereby an increased respiration rate is made possible. 'The acidity of the vacuole is due to the organic acid present. Whether the apple remains on the tree or is gathered, the acidity of the vacuole steadily falls, from the beginning of the cell-enlargement stage, at approximately the same rate. This explains why the climacteric rise occurs nearly at the same time, irrespective of date of gathering, since the acidity falls in any case, at about the same time, to a certain critical level at which the climacteric occurs. Other evidence has been obtained that the acidity of the vacuole is the main factor in determining the onset of the climacteric.' To what extent the pre-climacteric rise in internal  $O_2$  concentration, as indicated in the previous section and Fig. 15, is in turn the underlying factor determining such acidity changes, and therefore the basic stimulus to the initiation of ripening would appear to merit careful analysis. A number of experiments conducted by Kidd and West (14) indicate an important relationship between the incidence of the climacteric and the oxygen supply:

'When Bramley's Seedling apples in the late pre-climacteric stage were exposed to an atmosphere of pure oxygen at  $18^\circ C.$ , the incidence of the climacteric was markedly accelerated.

'Atmospheres containing 50 per cent. of oxygen brought about an earlier onset of the climacteric than does air, but with this percentage of oxygen the reaction was not an immediate one. Subnormal concentrations of oxygen delayed the onset of the climacteric; for instance, in 5 per cent. oxygen, not only was the onset of the climacteric rise in respiratory activity definitely retarded, but its magnitude was also greatly reduced. Results of a similar nature as regards the effect of subnormal oxygen upon the climacteric in pears have already been recorded.

'The results of certain experiments with apples exposed to an atmosphere from which oxygen was, as far as possible, eliminated, suggests that this gas plays an essential part in bringing about the changes which are responsible for the climacteric. It is found, for example, that if an apple in the pre-climacteric stage is kept in an atmosphere of nitrogen (0.3 per cent. or less of oxygen present), treatment with ethylene has no effect upon its respiratory activity, and when the apple is returned to air it is found to be still in the pre-climacteric condition. If the apple is then again treated with ethylene, the climacteric rise in respiratory activity is immediately induced, as already described.'

The reason for the decline in respiration rate during senescence is likewise obscure. Various explanations have been put forward. Black-

man (2) has advanced the view that the post-climacteric decline in respiration rate is in the nature of a starvation phenomenon being referable to the utilization of all the normal substrate of respiration. On the grounds of chemical analysis, this view has been refuted by Kidd and West (13), working on the apple, and by Gustafson (7) for the tomato; in both instances it was shown that the post-climacteric phase is characterized by an abundance of the carbohydrates normally thought to be involved in the respiratory process. Gustafson has attempted to account for the post-climacteric decline in respiration rate in such general terms as 'the whole system is slowly running down'. Kidd (16) has advanced the view that it is the 'rate of living' which kills, and pictures the metabolic machine as breaking down not from lack of fuel, but from wear and tear.

Reviewing the information available for the apple, Kidd (16) states that during senescence a slow decline in respiration takes place, accompanied by an increased production of aldehyde and alcohol. He suggests that this is due to diminishing activity of the system for the oxidation of aldehyde by molecular oxygen. The evidence from the present investigations, however, would seem to indicate that the ultimate causal factor is the inability of sufficient supplies of  $O_2$  to gain access to the tissues. Furthermore, the fact that with ageing, apples become increasingly sensitive to lack of  $O_2$  seems likewise explicable—in terms of gradients of gaseous concentration—when internal gas concentrations during senescence are duly considered.

As ripening advances important changes take place in the physical and chemical constitution of the flesh, with concomitant softening of tissues, an increase in 'wateriness' and final disintegration. Accompanying these modifications, it may be inferred that there are important changes in the condition of water in the tissues, the precise nature of which remains to be determined. The outstanding fact which has been established is that these changes make for a greater resistance on the part of the fruit flesh to the movement of gases. In the papaw, the internal concentration of oxygen continues to diminish rapidly during the post-climacteric period till finally anaerobic conditions are established. It is evident that metabolic processes are still operative during this period, but, in view of the greater resistance offered to the passage of gases, the cavity oxygen, utilized in metabolism, is not replaced, and hence a time comes when all the oxygen previously present in the cavity and intercellular spaces is used up. *Because of the importance of oxygen in normal metabolism, it is suggested that in this reduction of oxygen supply we have a causal factor which explains the 'running down' of the metabolic system, even though substrates for respiration are abundantly present.*

With regard to the transition from aerobic to anaerobic conditions during the later phase of senescence, Gustafson (9) has tentatively advanced

the hypothesis that some fruits, such as the tomato, may *normally*, i.e. in air, carry on intramolecular respiration, especially in the central tissues, where the oxygen tension is liable to be lower than in more superficially placed cells. As early as 1896 Gerber (5) observed that intramolecular respiration was characteristic of the ripening of various fleshy fruits, and indicated the possibility of a connexion between this and the formation of esters. A more recent study of tomatoes by Gustafson (8) has shown that there is considerable alcohol present in fruits maintained in air: this is at its maximum in nearly ripe, orange-red fruits; in green fruits the amount of alcohol increases as the fruits enlarge.

#### VIII. SUMMARY.

1. Respiration and transpiration rates have been studied in developing fruits, attention being paid to an evaluation of the operation of the size-factor in these processes.

2. By selecting large, hollow, fleshy fruits, e.g. the papaw, it has been possible to determine accurately and by simple means the internal concentrations of carbon dioxide and oxygen throughout development. The relation between such concentrations and the phenomenon of respiration as usually understood is discussed with special reference (i) to the resistance which tissues may offer to the movement of gases, and (ii) to the retentive capacity for  $\text{CO}_2$  which tissues at different stages of development may possess.

3. The phenomenon of the climacteric rise in ripening fruits is considered in detail, attention being directed to the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$  during that phase. It has been demonstrated that the curve for internal  $\text{CO}_2$  concentration (i) begins to rise prior to the onset of the climacteric and (ii) thereafter rises steadily and does not show any modification, comparable with the peak value, observed in 'external' respiration studies.

4. The post-climacteric phase, characterized by a decline in the rate of 'external' respiration, is marked by a progressive decrease in internal  $\text{O}_2$  concentration till finally anaerobic conditions are established.

5. The fundamental part played by  $\text{O}_2$  throughout development and ripening would appear to be substantiated by these studies. (i) the onset of ripening is coincident with the peak value of internal  $\text{O}_2$  concentration during development; (ii) the failure of  $\text{O}_2$  to gain access to the flesh tissues during the later stages of ripening is thought to be responsible for the phenomenon of senescence in fruits.

The writers have much pleasure in expressing their deep indebtedness to Professor V. H. Blackman, F.R.S., for his valuable criticism of this work.

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# Studies in Tropical Fruits.

## II. Observations on Internal Gas Concentrations in Fruit.

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With eleven Figures in the Text.

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### I. INTRODUCTION.

IN the first paper of this series (5) it was shown that certain large fleshy tropical fruits are well suited to the investigation of internal concentrations of carbon dioxide and oxygen. Thus it became possible to ascertain the relation between the superficial liberation of CO<sub>2</sub> during respiration and the concurrent internal concentration of that gas. It was further demonstrated that the rate of liberation of CO<sub>2</sub> externally depends on the state of development or maturity of the fruit and on the resistance which the fleshy tissues and epidermis offer to the movement of gases. In the present paper an account is given of further investigations which show in what manner and to what extent internal gas concentrations are modified under conditions involving the use of controlled external atmospheres. Such observations are of importance in relation to gas-storage of fruit.

[*Annals of Botany*, Vol. L. No. CXCIX. July, 1936.]



The data relate to the changes which take place in the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$ , (i) when the atmosphere surrounding the fruit is varied in respect of these two gases, and (ii) when the normal atmosphere is replaced by pure nitrogen.

## II. MATERIALS AND METHODS.

For the direct measurement of internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$  large fleshy fruits such as the papaw, water-melon, pumpkin, and cucumber, i.e. fruits possessing either a cavity or an extensive system of intercellular air-spaces, were used. The method of obtaining samples for analysis by the Haldane apparatus has already been described (5). For convenience this may be briefly restated here: using a sterilized cork-borer, 3/16-inch diameter, a plug of tissue was removed from the fruit; thereafter, without loss of time, a glass tube of suitable diameter, fitted with a vaselined washer and clipped tube, was inserted; the tube was then attached to the gas-analysis apparatus, the clip opened, and a sample of internal gas withdrawn. As a rule no difficulty was experienced in obtaining such samples.

## III. PRODUCTION OF HIGH INTERNAL CONCENTRATIONS OF $\text{CO}_2$ BY SMEARING WITH VASELINE.

Anticipating that considerable changes in the internal concentration of  $\text{CO}_2$  could be induced by the simple process of smearing fruits with vaseline, full-grown green and colouring papaws were fitted with tubes as described and maintained at a constant temperature of  $70^\circ\text{F}$ ., the respective initial internal  $\text{CO}_2$  concentrations were ascertained and estimations continued at intervals after the fruits had been subjected to a light but thorough superficial application of vaseline. Internal  $\text{CO}_2$  concentrations were also determined in untreated fruits from time to time to serve as controls. Typical results are illustrated in Fig. 1, curves for the normal internal  $\text{CO}_2$  during ripening being shown for contrast. In the vaselined green papaw the internal  $\text{CO}_2$  curve rose from its low initial value to a concentration of 14.8 per cent. in twenty-four hours. Measurements made on subsequent days afforded a leakage curve as indicated.<sup>1</sup> When the  $\text{CO}_2$  concentration had fallen to 5.6 per cent. the fruit was heavily vaselined and air admitted into the fruit cavity by opening the clipped tube. Subsequent analyses of internal  $\text{CO}_2$  showed a gradual rise to a concentration of 15.8 per cent., followed by a slow decrease. In a more mature fruit showing the first traces of ripening colour but with the internal concentration of  $\text{CO}_2$

<sup>1</sup> Under laboratory conditions in Trinidad vaseline quickly dries into the fruit skin. This may, in part, account for the leakage recorded. On the other hand, the influence of the decreased oxygen supply on metabolism, together with increased resistance to the outward diffusion of  $\text{CO}_2$ , afford an adequate explanation of the results observed.

similar to that of the green fruit, the vaseline treatment caused a rise in internal  $\text{CO}_2$  concentration to 23.4 per cent. in twenty-seven hours. This was followed by a decrease in concentration and, after four days, by the premature appearance of anthracnose spots (5). These observations are in

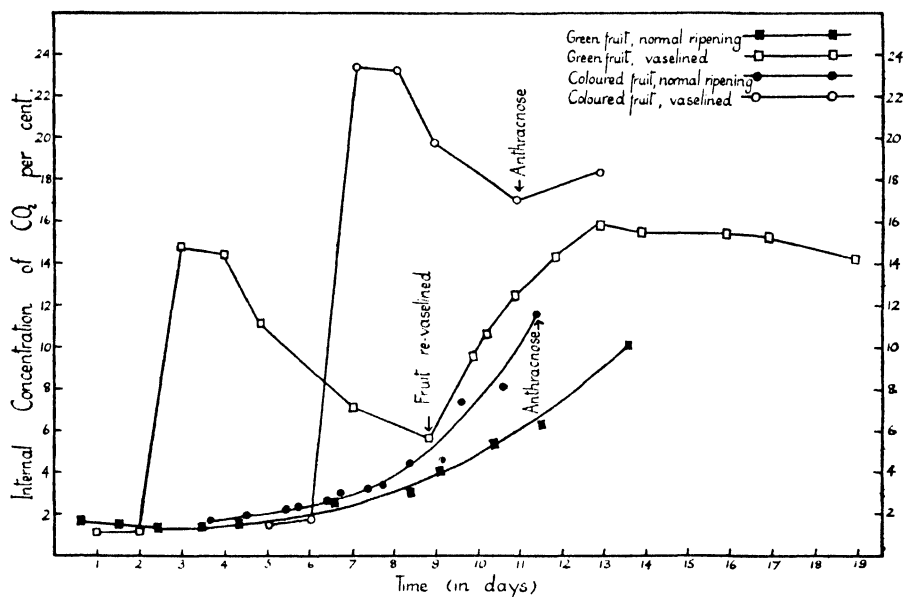


FIG. 1. Changes in the internal concentrations of  $\text{CO}_2$  in green and colouring papaws as a result of superficial smearing with vaseline.

sharp contrast to the gradual increase in internal  $\text{CO}_2$  concentration during normal ripening.

A small pumpkin, diameter 17 cm. with initial internal  $\text{CO}_2$  and  $\text{O}_2$  concentrations of 3.0 per cent. and 17.0 per cent. respectively, was heavily vasedined and held at  $70^\circ\text{F}$ . In the course of eight days the  $\text{CO}_2$  concentration rose gradually to 23.52 per cent. while oxygen was reduced to 0.20 per cent. During the next four days the  $\text{CO}_2$  concentration continued to increase. The fruit appeared normal externally, but on cutting was found to have undergone a type of physiological breakdown.

A cucumber, with an initial internal  $\text{CO}_2$  concentration of 2.0 per cent., when vasedined and held at  $70^\circ\text{F}$ . showed a rise in concentration to 22.96 per cent.  $\text{CO}_2$  in twenty hours. A few days later the fruit developed anthracnose spots prematurely.

#### IV. THE RELATION BETWEEN THE INTERNAL CONCENTRATION OF $\text{CO}_2$ AND CHANGES IN THE EXTERNAL ATMOSPHERE.

In some preliminary experiments attempts were made to estimate the rate of liberation of  $\text{CO}_2$  from the papaw and other fruits, i.e. rate of

respiration as usually understood, as follows: a fruit of known weight and volume was placed in a gas-tight container of known volume and the  $\text{CO}_2$  liberated sampled at intervals by means of the gas-analysis apparatus. In experiments of this type it was found that the respiration rate showed

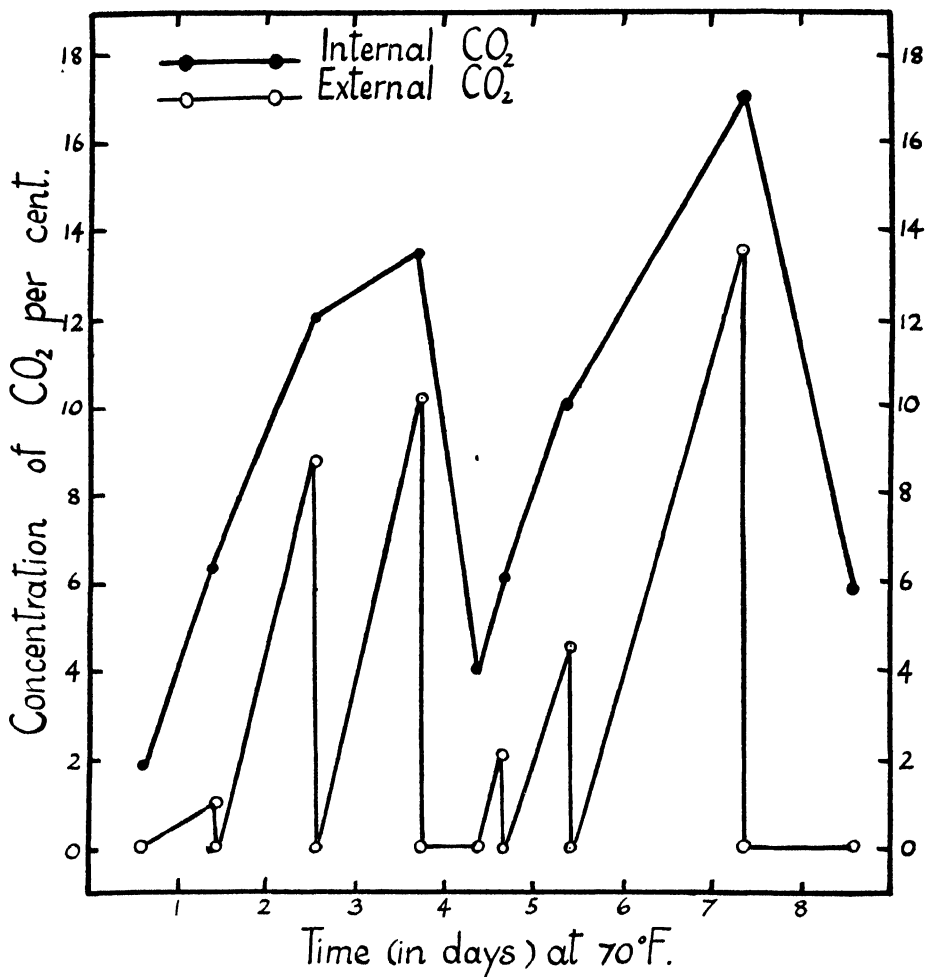


FIG. 2. Colouring papaw held in closed gas-container showing the relation between external concentration of  $\text{CO}_2$  and internal (fruit cavity) concentrations: vertical lines in the external  $\text{CO}_2$  curve indicate the recharging of the gas-container with air, horizontal lines show where the fruit was left exposed on the bench.

marked fluctuations. Thus on sampling after one hour the rate of respiration (mg. per kg. per hour) was always much greater than if the experiment had been extended over ten or twenty hours. Again, if, after a respiration experiment extending over twenty-four hours the gas-container was recharged with normal air, and the rate of respiration again determined after one hour, the second reading was invariably far in excess of the first.

The defects of this method, which, in the longer experiments involved the accumulation of considerable quantities of  $\text{CO}_2$  in the containing vessel, and, by inference, changes in the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$  in the fruit tissues and cavity, were soon realized. From the results obtained,

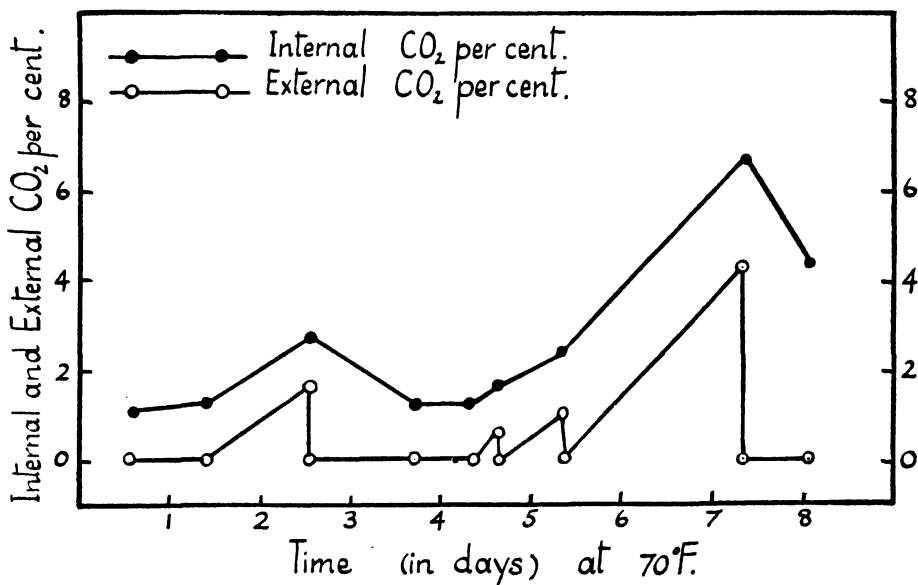


FIG. 3. Green papaw held in closed gas-container, showing the relation between external and internal (fruit cavity, concentrations of  $\text{CO}_2$ . Annotations as in Fig. 2.

however, an interesting line of experimentation was suggested, that of following the changes induced in internal gas concentrations in fruits by varying the external atmosphere.

In preliminary experiments the procedure adopted was as follows: A papaw fruit was fitted with a sampling tube and the initial internal concentration of  $\text{CO}_2$  ascertained. The fruit was then placed in a gas-tight container, and after some time had elapsed the concentration of the  $\text{CO}_2$  which had accumulated in the container was measured. The fruit was then immediately removed and its internal  $\text{CO}_2$  concentration also measured. Thereafter the fruit was allowed to remain exposed on the bench for some hours and the internal atmosphere again sampled; in some instances it was replaced in the container which had been recharged with normal air. Fig. 2 for a colouring fruit and Fig. 3 for a smaller green fruit from the same tree show the type of observation recorded; vertical portions of the 'external'  $\text{CO}_2$  concentration curves indicate where the container was recharged with normal air, and horizontal portions where the fruit on removal from the container was left exposed on the bench, i.e. with  $\text{CO}_2$  at a concentration of 0.03 per cent. The experiment was conducted at  $70^\circ\text{F}$ .; the data of Table I indicate the nature of the experimental material used.

The parallelism between the ascending curves for internal and external concentrations of  $\text{CO}_2$ , the former always showing higher values, and the rapid decrease in the internal concentration observed when fruits were removed from a high external concentration of  $\text{CO}_2$  to normal air, indicate

TABLE I.

	Weight (gm.).	Volume (c.c.).	Internal volume (c.c.).	Volume of container (litres).
Colouring fruit	2617	4455	1590	26
Green fruit	2083	3465	1150	27

how closely internal gas concentrations are related to the external gas environment. The importance that gradients of gaseous concentration assume in these circumstances is clearly shown. The relation between maturity of fruit and the response to the above experimental treatment is also seen by comparing Figs. 2 and 3.

Because of the value of the information derived from this type of experiment an attempt was next made to follow the process in greater detail. Accordingly a large subspherical papaw, showing ripening colour in the flesh and externally in the furrows, was fitted with a sampling tube and placed in a gas-tight container arranged with lead-off tubes so that the 'external' and 'internal' gases could be analysed as required without opening the container. The  $\text{CO}_2$  liberated during respiration was then allowed to accumulate inside the container and gas analyses undertaken throughout the duration of the experiment. On three successive occasions when both 'internal' and 'external'  $\text{CO}_2$  concentrations had reached high values the container was recharged with normal air, closed, and measurements immediately begun again. The results obtained at a temperature of  $80^\circ\text{F}$ . are shown in Figs. 4 and 5. Possible stimulating effects resulting from the accumulation of volatile substances, if present, could not be excluded in an experiment of this kind. It was not evident that unavoidable small fluctuations in temperature had seriously influenced the general trend of the experiment.

The following data indicate the type of fruit used and some of the more salient observations recorded:

Weight of fruit: 2,069 gm.	Surface area by calculation (taking 'mean diameter' as 19.4 cm.): 1,129 sq. cm.
Volume of fruit: 3,434 c.c.	Volume of atmosphere in container: 12,180 c.c.
Volume of internal cavity: 1,230 c.c.	Initial internal concentration of $\text{CO}_2$ : 3.85 per cent.
Thickness of flesh: 2.60 cm.	Initial internal concentration of $\text{O}_2$ : 16.65 per cent.
Diameter: 19.2 cm.	
Long axis: 19.6 cm.	
Surface area by measurement: <sup>1</sup> 1,109 sq. cm.	

At the beginning of the experiment the rate of respiration increased steadily, reaching a maximum value of 82.54 mg. per kg. per hour (Fig. 5).

<sup>1</sup> The method used was to gum on squared paper.

At this point the concentration of  $O_2$  in the internal cavity was 8.5 per cent. and that of the container 15.0 per cent. Thereafter the rate of liberation of  $CO_2$  from the fruit decreased slightly, a comparatively steady rate being finally maintained after approximately twenty-four hours.

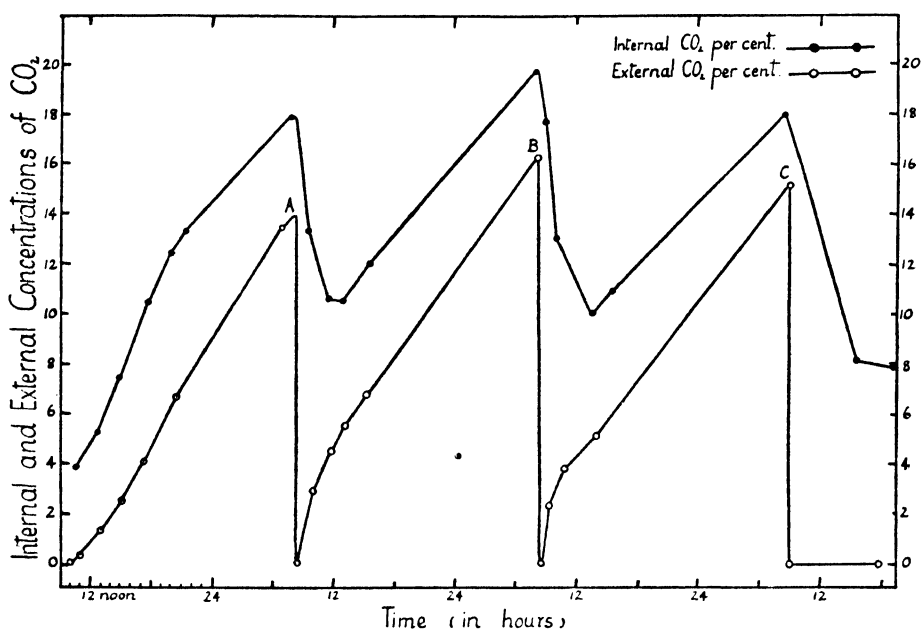


FIG. 4. Papaw held in closed gas-container, showing the relation between external and internal (fruit cavity) concentrations of  $CO_2$ . At A, B, and C on the external  $CO_2$  curve the gas-container was recharged with air. (See also Fig. 5.)

During this period the sum of the percentages of internal  $CO_2$  and  $O_2$  was approximately 21 per cent.

When the internal  $CO_2$  concentration had risen to 17.92 per cent. (A, Figs. 4 and 5), the container was opened and recharged with normal air. During the following hour the  $CO_2$  stored up within the fruit diffused out with great rapidity so that a high 'rate of respiration', i.e. superficial liberation of  $CO_2$  (193.4 mg. per kg. per hour) was observed. Oxygen likewise diffused rapidly inwards replacing the outwardly diffusing  $CO_2$ . The sum of the internal concentrations of  $CO_2$  and  $O_2$  at this stage showed a considerable increase over 21 per cent., an observation which might be regarded as suggesting that the normal utilization of  $O_2$  was not taking place. A more likely explanation is that this rise in the percentage  $CO_2$  plus  $O_2$  represents the rapid liberation into the cavity of  $CO_2$  from the highly charged flesh tissues. The measurements of internal oxygen indicated that  $CO_2$  liberated externally did not represent normal metabolic activity until the concentration of  $O_2$  had risen to approximately 12 per cent.

This observation was confirmed later in the course of the same experiment (Fig. 5).

When the internal concentration of  $\text{CO}_2$  had later been allowed to rise to 19.69 per cent. followed by recharging of the container with normal air,

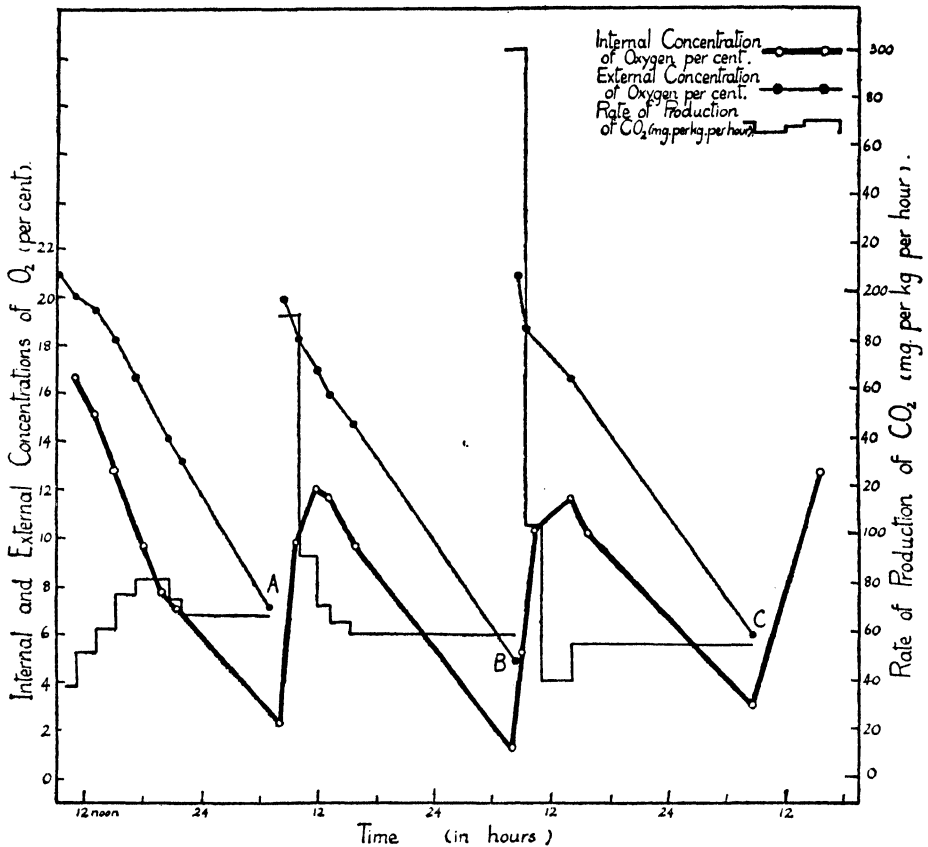


FIG. 5. Rate of respiration, and external and internal (fruit cavity) concentrations of  $\text{O}_2$ .  
(Explanation as in Fig. 4.)

$\text{CO}_2$  was liberated from the fruit at the rate of 301.7 mg. per kg. per hour (point B, Figs 4 and 5). Again, under the conditions of the experiment, the high 'respiration rate' is seen to be of relatively brief duration and is directly explicable in terms of the rapid outward diffusion of  $\text{CO}_2$  in relation to the abnormally high internal concentrations which had been induced.

For purposes of reference, some 'respiration rates' recorded as c.c. per hour per sq. cm. may be recorded. During the first part of the experiment values ranged from 0.041 to 0.086 (maximum) c.c. per sq. cm. per hour, reaching a steady rate of 0.071 c.c. per sq. cm. per hour. After recharging

the container with normal air, a 'respiration rate' of 0.202 c.c. per sq. cm. per hour (with internal  $\text{CO}_2$  = 17.92 per cent.) was recorded; on the second occasion this was calculated to be 0.315 c.c. per sq. cm. per hour (with internal  $\text{CO}_2$  of 19.69 per cent.).

In order to eliminate the effect of metabolic changes associated with the climacteric phase, the experiment was repeated at 70° F., using a full-grown, green papaw (from the same tree) whose flesh was still white and free from any trace of ripening colour.

The following data may be cited :

Weight of fruit : 1,747 gm.  
Volume of fruit : 2,795 c.c.  
Volume of internal cavity : 980 c.c.  
Diameter : 17.2 cm.  
Long axis : 18.6 cm.  
Surface area by measurement : 1,005 sq. cm.

Initial internal concentration of  $\text{CO}_2$  : 1.50 per cent.  
Initial internal concentration of  $\text{O}_2$  : 19.00 per cent.  
Fruit green, flesh white.

As before, the fruit fitted with a sampling tube was placed in a gas-tight container and the  $\text{CO}_2$  liberated during respiration estimated from time to time, together with contemporaneous determinations of the concentrations of  $\text{CO}_2$  and  $\text{O}_2$  within the fruit cavity. On two occasions, when high external  $\text{CO}_2$  values had been attained, the container was opened and recharged with normal atmosphere. The results are illustrated graphically in Figs. 6 and 7.

Over a period of approximately 118 hours from the beginning of the experiment respiration proceeded at a slowly declining rate under the self-created gas-storage conditions. When the container atmosphere reached a  $\text{CO}_2$  concentration of 15.12 per cent. (and  $\text{O}_2$  of 4.38 per cent.) the internal cavity of the papaw showed a  $\text{CO}_2$  concentration of 16.17 per cent. (and  $\text{O}_2$  = 3.19 per cent.). The almost parallel but slightly convergent course followed by the two curves for increasing concentration of  $\text{CO}_2$  (internal and external) is clearly shown in Fig. 6.

On replacing the atmosphere of the container with normal air (point A, Figs. 6 and 7), very rapid adjustments of  $\text{CO}_2$  and  $\text{O}_2$  concentrations were recorded in response to the existing high gradients of gaseous concentration,  $\text{CO}_2$  passing from the fruit flesh and cavity into the container,  $\text{O}_2$  from the container into the fruit. The rapid decrease in the internal concentration of  $\text{CO}_2$  is reflected in the initial high 'respiration rates', (Fig 7, point A); the 'respiration rate' rapidly returned to approximately its former value.

Prior to recharging the container with normal atmosphere (point A) the sum of the concentrations of  $\text{CO}_2$  and  $\text{O}_2$  within the fruit cavity was 19.36 per cent. and for the container atmosphere 19.50 per cent. During the transition period following the recharging of the container with normal air, the sum of the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$  rose quickly to



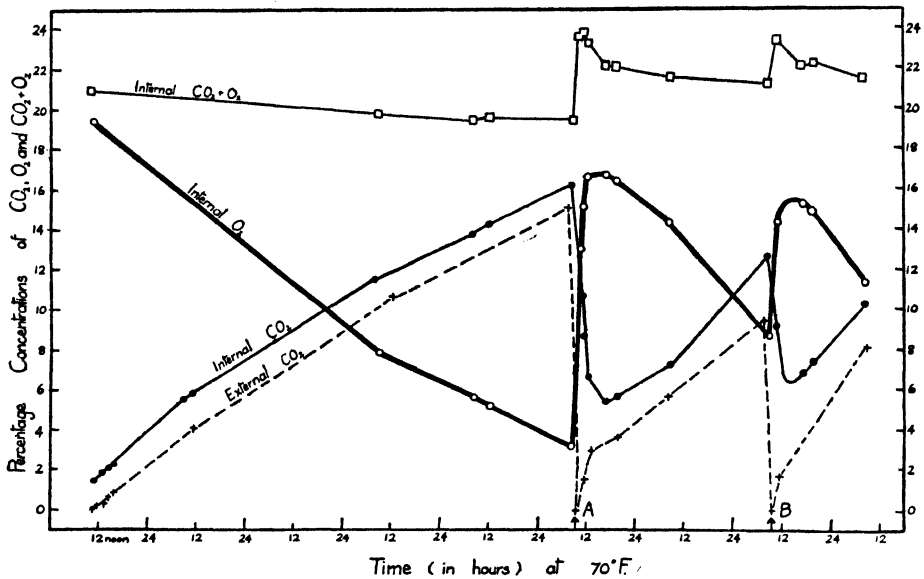


FIG. 6. Full-grown, green papaw enclosed in gas-container. Curves showing external and internal (fruit cavity) concentrations of  $\text{CO}_2$ , internal concentration of  $\text{O}_2$  and of  $\text{CO}_2 + \text{O}_2$ . The gas-container was recharged with normal air at A and B. (See also Fig. 7.)

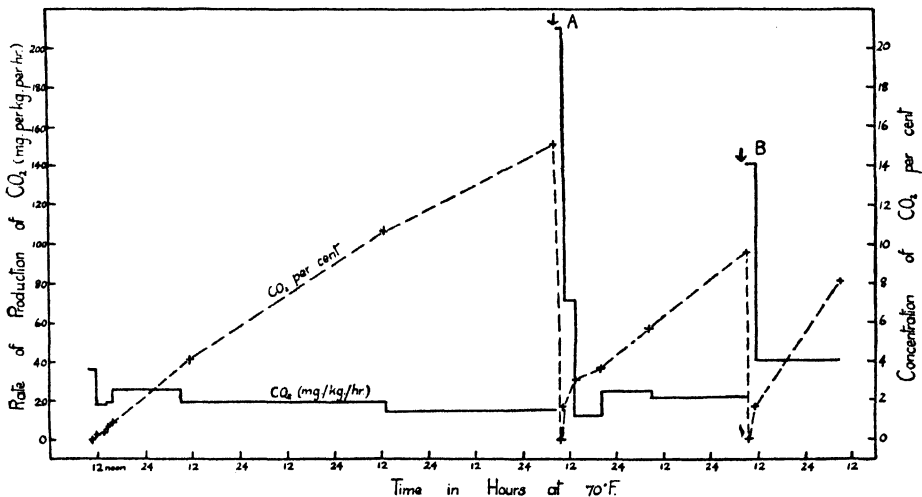


FIG. 7. Full-grown, green papaw enclosed in gas-container. Curves showing external concentration of  $\text{CO}_2$  and rate of respiration. (Compare with Fig. 6.)

23.65 per cent. and thereafter declined to 21.25 per cent.; the sum of the concentrations of  $\text{CO}_2$  and  $\text{O}_2$  in the container lay between 21 and 22 per cent. The high transition values of  $\text{CO}_2$  plus  $\text{O}_2$  within the fruit cavity,

recorded also in the previous experiment, indicate the rapid liberation of  $\text{CO}_2$  from the highly charged flesh tissues into the cavity and are not referable to immediate metabolism; to what extent they also indicate the slower utilization of  $\text{O}_2$  by tissues still saturated with  $\text{CO}_2$  cannot be determined. The gradual reduction internally and externally in the value of  $\text{CO}_2$  *plus*  $\text{O}_2$  prior to recharging the container, as noted above, is evidently related to the accumulation of  $\text{CO}_2$  in the flesh tissues.

After a further forty-eight hours, during which the  $\text{CO}_2$  in the container rose to 9.56 per cent., and that in the cavity to 12.63 per cent., the container was again recharged with normal air (point B, Figs. 6 and 7). Essentially similar results, involving a high rate of liberation of  $\text{CO}_2$  externally and readjustment of the concentrations of  $\text{CO}_2$  and  $\text{O}_2$ , were obtained. At this stage, as the fruit had been held at  $70^\circ\text{F}$ . for seven days, indications of ripening were observed, with a concomitant increase in the normal respiration rate in air.

In the previous experiment, using a ripening fruit at  $80^\circ\text{F}$ ., it was observed that following the creation of gas-storage conditions, normal utilization of  $\text{O}_2$  was not resumed until the internal concentration was readjusted to a value of approximately 12 per cent. In the present experiment, using a green fruit at  $70^\circ\text{F}$ ., the corresponding oxygen value lay between 15.5 and 17.0 per cent.

### *Discussion.*

The experiments described in this section indicate: (i) the close relation that exists between the internal concentrations of carbon dioxide and oxygen in a fruit and the gaseous atmosphere by which it is surrounded; (ii) the importance of gradients of gaseous concentration in determining the 'rate of respiration', i.e. liberation of  $\text{CO}_2$  at the surface of the fruit; (iii) that under certain experimental conditions the rate of respiration, as ascertained by measuring the amount of  $\text{CO}_2$  liberated superficially, may bear no direct relation to the rate at which cellular respiration is proceeding at that time; (iv) the rapidity with which readjustments of gaseous concentration may take place; (v) the importance of the  $\text{CO}_2$  present in the flesh in relation to the measurement of respiration.

Experiments of a somewhat similar nature to those outlined in this section have been described by Willaman and Beaumont (6). These workers studied the respiration of apple twigs, potato tubers, and wheat grains by allowing the  $\text{CO}_2$  of respiration to accumulate in a closed gas chamber. Under such conditions the rate of  $\text{CO}_2$  production was observed to decrease continuously with time. When aspiration of the gas container

was begun the rate of respiration immediately assumed much higher values than before; they consider that the magnitude of these values may possibly be proportional to the amount of  $\text{CO}_2$  previously accumulated in the tissues. Several hours elapsed before a constant respiration rate was again attained. According to Willaman and Beaumont: 'One explanation of this phenomenon is that we are merely observing an equilibrium between the  $\text{CO}_2$  in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess of  $\text{CO}_2$  in the latter is removed but slowly when aspiration is commenced. Another possible explanation is that the accumulation of  $\text{CO}_2$  in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point and hence increases the permeability of the protoplasm; and that this is responsible for an actual increase in rate of  $\text{CO}_2$  production. The evidence in the literature on the pH of cell-sap and on the iso-electric points of plant proteins bears out this view to a certain extent. Direct evidence in its favour was secured by passing HCl gas into a respiration chamber containing wheat grain. A duplicate of the  $\text{CO}_2$  effect was obtained. Although admitting that the proof for the latter explanation is still far from complete, the writers nevertheless subscribe to it, and offer it for the criticism of others.'

From the data submitted in the present paper, where it has been possible to measure not only the rate of liberation of  $\text{CO}_2$  at the fruit surface but also to observe simultaneously the internal and external readjustments of the several gases, it would appear that the high internal concentrations of  $\text{CO}_2$  (whether in the intercellular air-spaces, fruit cavity or cells) resulting from the procedure adopted, accounts satisfactorily for the high 'respiration rates' observed when the gas container is aspirated or immediately recharged with normal air.

In the matter of retentive capacity for  $\text{CO}_2$ , Gerhardt and Ezell (2) have shown that  $\text{CO}_2$  is absorbed to a much greater extent by the tissues of pears than by those of apples. On removing fruits from an atmosphere containing 35 per cent.  $\text{CO}_2$ , the concentration of that gas in the intercellular spaces of these fruits was found to decrease with great rapidity (approximately 70 per cent. was lost in eight hours) till normal concentrations, as in fruit kept in air, were regained.

#### V. RATE OF RESPIRATION AND INTERNAL GAS CONCENTRATIONS UNDER ANAEROBIC CONDITIONS.

Again using the papaw, three experiments were undertaken in which observations were made on 'rate of respiration' and changes in internal gas concentrations when fruits were deprived of normal air and held in an atmosphere of nitrogen. While the methods adopted are susceptible of

considerable improvement, the results of the three experiments taken in conjunction yield evidence which greatly simplifies certain aspects of the complex phenomenon of anaerobic respiration.

(i) *Nitrogen supplied externally with subsequent accumulation of CO<sub>2</sub>.*

Using the experimental method outlined in Section IV, measurements of the internal concentrations of CO<sub>2</sub> and O<sub>2</sub> and of 'respiration rate' were made when the gas-container was initially supplied with pure nitrogen.

The procedure was briefly as follows: a large papaw was enclosed in a gas-tight container and the initial internal concentrations of CO<sub>2</sub> and O<sub>2</sub> and the rate of superficial liberation of CO<sub>2</sub> measured. Pure nitrogen, from a gas cylinder tested for O<sub>2</sub> and heated to room temperature (85° F.) by being passed through a metal coil immersed in suitably warm water, was then supplied rapidly to the gas-container for ten minutes till its atmosphere on analysis held O<sub>2</sub> at a concentration of less than 1 per cent. The nitrogen supply was then turned off and the gas-container closed. Thereafter, determinations of the concentrations of CO<sub>2</sub> and O<sub>2</sub> in the fruit cavity and in the container were made, the CO<sub>2</sub> liberated by the fruit during respiration being allowed to accumulate in the container. These measurements were made at intervals for approximately twenty-eight hours; during this time the continuation of metabolic processes, with concomitant production of CO<sub>2</sub>, resulted in the accumulation of this gas in the container and in the fruit cavity; thus when the external concentration of CO<sub>2</sub> had reached a value of 10.5 per cent., the corresponding internal value was 12.35 per cent. The container was then recharged with normal air, closed, and measurements continued. The complete experimental record is illustrated graphically in Fig. 8.

The following data may be cited:

Weight of fruit: 3136.5 gm.	Initial rate of respiration: 47.40 mg. per kg. per hour.
Volume of fruit: 4,210 c.c.	Initial internal concentration of CO <sub>2</sub> : 4.9 per cent.
Volume of fruit cavity: 925 c.c.	Initial internal concentration of O <sub>2</sub> : 16.0 per cent.
Fruit ripening, yellow in furrows.	Concentration of O <sub>2</sub> in container at beginning: 0.91 per cent.
Volume of gas container: 16,490 c.c.	
Volume of atmosphere: 12,200 c.c.	
Temperature of experiment: 85° F. (29.5° C.).	

The outstanding features of this experiment were:

1. The great rapidity with which oxygen, originally present in the fruit cavity at a concentration of 16 per cent., diffused outwards into the container, in relation to the new concentration gradient (16—1 per cent.) established by irrigating the gas-container with N<sub>2</sub>; in other words, the initial phase of 'anaerobic' respiration, under such experimental conditions involves a preliminary respiration of O<sub>2</sub>.

2. 'Respiration rate', i.e. the rate at which CO<sub>2</sub> was liberated externally

showed initial high values.<sup>1</sup> This was followed by a rapid decline till an approximately steady rate, lower than the rate of respiration in air at the commencement of the experiment, was established; this latter portion of the curve represents the 'rate of respiration' under anaerobic conditions in conjunction with progressive accumulations of  $\text{CO}_2$  externally and internally.

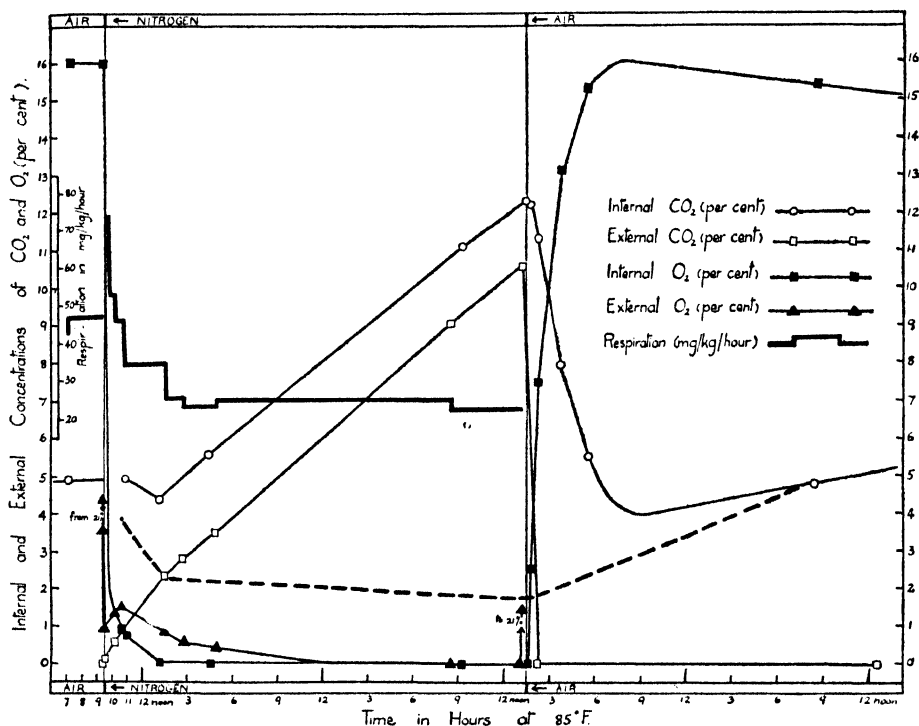


FIG. 8. Full-grown, colouring papaw in gas-container filled with nitrogen at 9.30 a.m. atmosphere replaced with air at 12 noon on following day. Curves showing internal and external concentrations of  $\text{CO}_2$  and  $\text{O}_2$  and also rate of respiration. (See text.)

3. During the anaerobic phase, the rise in the internal concentration of  $\text{CO}_2$ , as in previous experiments in closed gas-containers, was governed by the increasing concentration of  $\text{CO}_2$  externally. Accordingly the data for internal and external  $\text{CO}_2$  concentrations yield ascending, slightly converging curves. If allowance is made for this, the actual curve for internal  $\text{CO}_2$  concentration, had all the  $\text{CO}_2$  been removed from the container atmosphere, would have been approximately that indicated by the discontinuous line in Fig. 8, obtained by subtracting external from internal concentrations at equivalent points in time; in other words, the ultimate effect of placing the fruit under anaerobic conditions was to decrease the rate of cellular respiration, and in consequence the internal concentration of  $\text{CO}_2$ .

<sup>1</sup> Actually, in this experiment, the maximal value was not recorded, because of the manner in which  $\text{N}_2$  was supplied.

4. On recharging the container with normal atmosphere, rapid diffusion of  $O_2$  inwards and of  $CO_2$  outwards (again affording high transitional 'respiration rates' not shown in Fig. 8 but similar to those recorded in Figs. 5 and 7) was again observed, the internal concentrations of these gases eventually attaining values approximating to those observed at the commencement of the experiment.

5. The relatively slow readjustment of the internal  $CO_2$  concentration (the discontinuous line in Fig. 8) to its normal value in air is explicable in terms of (a) the initial lag intervening until metabolic processes have regained their characteristic intensity for normal atmosphere, and (b) the time required to accumulate in the large fruit cavity and tissues the amount of  $CO_2$  characteristic, under normal conditions, of the fruit's state of ripeness.

(ii) *Nitrogen supplied internally and externally with subsequent accumulation of  $CO_2$ .*

After a period of two days, during which the fruit used in the preceding experiment had been left exposed to the normal atmosphere, a further experiment under anaerobic conditions was carried out. The fruit showed increased ripening, but otherwise, as regards respiration rate and internal gas concentrations, was apparently normal.

In the experiment to be detailed, the *fruit cavity* was first of all irrigated thoroughly with nitrogen; immediately afterwards the gas-container was also thoroughly irrigated with nitrogen, whereby the  $O_2$  concentration was reduced from 21 per cent. to 0.64 per cent. The gas-container was then closed, and the  $CO_2$  of respiration allowed to accumulate as before. Fig. 9 shows the 'rate of respiration' and the internal and external gas concentrations measured from time to time.

The first irrigation, internal and external, with  $N_2$  was carried out from 9.19 to 9.30 a.m.; at 2.45 p.m. the container was again thoroughly irrigated for fifteen minutes with  $N_2$  so that the  $CO_2$  concentration was reduced to 0.05 per cent., and the container again closed and measurements continued.

The following points are noteworthy:

1. Notwithstanding the fact that the  $CO_2$  was removed from the fruit cavity by irrigating with nitrogen, the transition to anaerobic conditions was again marked by an increased 'respiration rate' for this gas; this rapidly fell to a lower value than the original rate in air; finally, a declining but approximately steady value was reached, attributable to the combined influence of the absence of oxygen and the progressive accumulation of  $CO_2$  in the container atmosphere.

2. Although the internal cavity was thoroughly irrigated for five minutes with a rapid flow of nitrogen (from 9.19 to 9.24 a.m.)—a period of time which previous experience indicated was more than sufficient to

discharge all the gas originally present in the cavity—the first determination of internal gas concentrations made at 9.31 a.m. showed that  $O_2$  had been reduced from 14.26 per cent. to 1.43 per cent., and  $CO_2$  from 7.35 per cent. to 2.60 per cent. In other words, whereas the first had been reduced to

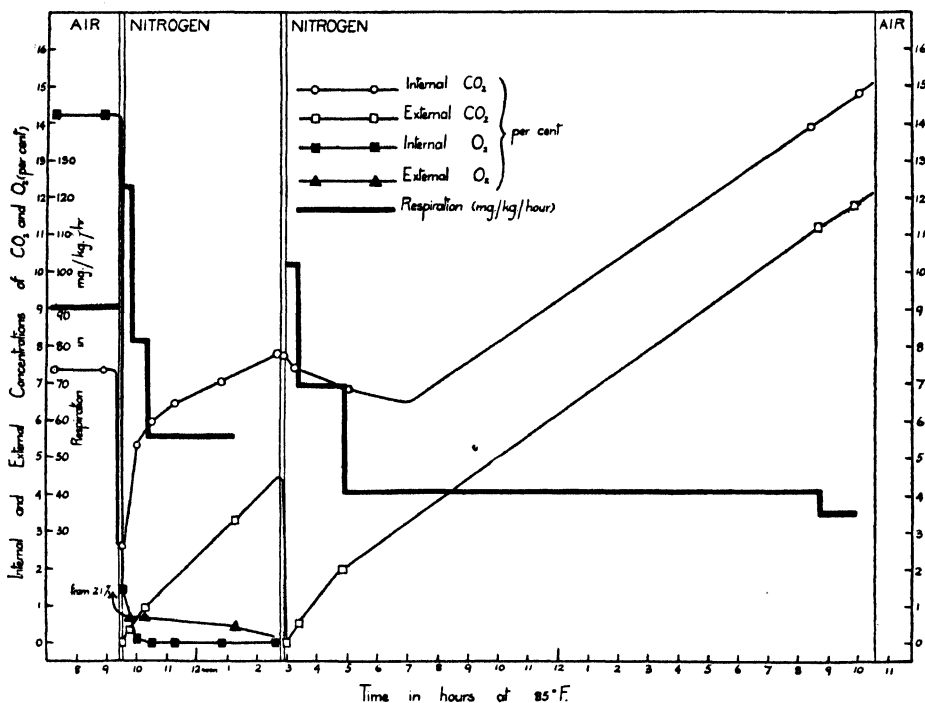


FIG. 9. Papaw, the same as that of Fig. 8 but slightly more ripe, in gas-container thoroughly irrigated with nitrogen at 9.30 a.m. approximately; gas-container again recharged with pure nitrogen at 2.45 p.m. Respiration rates, internal and external  $CO_2$  and  $O_2$  concentrations.

one-tenth of its original concentration, the second had only been reduced to approximately one-third. From these considerations it is evident that the concentrations observed represent quantities of these gases which, originally present in the flesh tissues, have diffused into the fruit cavity as a result of changes in concentration gradient from flesh to cavity induced by the introduction of nitrogen into the latter. Confirmation of this explanation is seen in the further increase in internal  $CO_2$  concentration, to 5.30 per cent., at 9.58 a.m.

3. Allowing for the effect of the accumulation of  $CO_2$  externally during the experiment, it is seen that anaerobic conditions cause a reduction in cellular respiration; hence internal  $CO_2$  concentration is subnormal with concomitant reduction in the rate at which that gas is liberated superficially.

The curves in Fig. 10, derived from the data in Fig. 9 and discussed

above, show corrected values for internal  $\text{CO}_2$  concentrations and observed 'respiration rates' (superficial liberation of  $\text{CO}_2$ ) for the period during which the fruit was maintained under anaerobic conditions. The close parallelism between the two curves is to be noted. Two peak values are shown on the internal  $\text{CO}_2$  concentration curve: the first, A, indicates the point at which

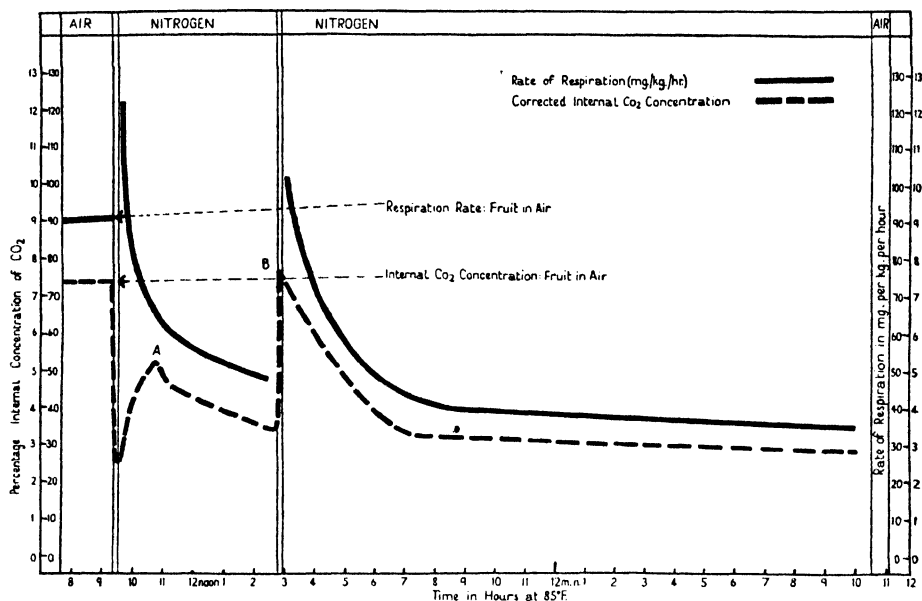


FIG. 10. Respiration in nitrogen showing relation between internal concentration of  $\text{CO}_2$  and rate of respiration. (Cf. text and Fig. 9.)

$\text{CO}_2$ , coming out of the flesh tissues into the cavity after irrigation with  $\text{N}_2$ , reaches equilibrium with the rate of external liberation of  $\text{CO}_2$ ; the second, B, is due solely to the accumulation of  $\text{CO}_2$  in the fruit cavity in relation to the experimental procedure adopted. At both points, A and B, high transitional 'rates of respiration' were recorded, the same steady low rate being eventually attained.

As the internal concentration of  $\text{CO}_2$ , in conjunction with the resistance which tissues offer to the movement of gases, is the factor controlling 'respiration rate' (5), the data submitted in experiments (i) and (ii) above appear to offer conclusive proof: (1) that, in the fruit under consideration, anaerobic conditions reduce the 'rate of respiration' to approximately one-half the rate in air, and (2) that the initial high 'respiration rates' recorded by F. F. Blackman and other workers, when a fruit is placed in an atmosphere of nitrogen, are not necessarily an indication that metabolism is proceeding at an increased rate: they are transitional values only, explicable in terms of (a) the amount of  $\text{CO}_2$  normally retained by the flesh tissues (varying according to the stage of development or ripeness of the



fruit), and (b) changes induced in this retentive capacity by the removal of oxygen.

(iii) *Nitrogen supplied externally, with removal of CO<sub>2</sub>.*

From the data derived from experiments (i) and (ii) above, it may be deduced that the effect of transferring a fruit from normal air to nitrogen is

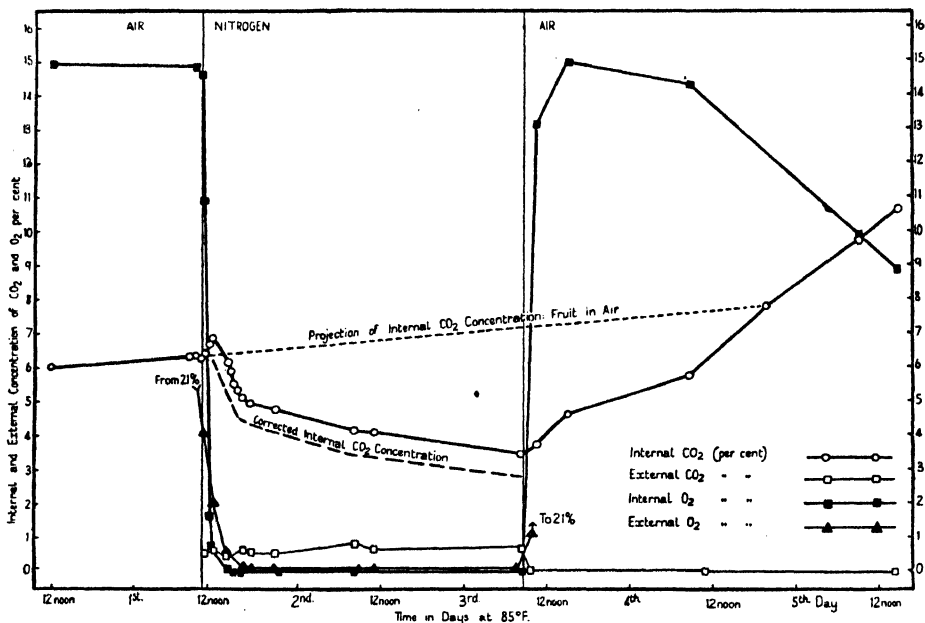


FIG. 11. Papaw in gas-container continuously irrigated with nitrogen. (See text.)

ultimately to bring about a marked reduction in the rate at which metabolism proceeds. Both 'rate of respiration' and the concentration of internal CO<sub>2</sub> which controls it, are, in fact, reduced to values lower than those for normal atmosphere. In both experiments, however, the direct effect of allowing respiration to proceed in nitrogen was obscured by the procedure adopted, i.e. the CO<sub>2</sub> liberated was allowed to accumulate in the gas-container. In the experiment now to be detailed the influence of the latter factor was almost eliminated so that direct readings of internal CO<sub>2</sub> concentrations, under anaerobic conditions, were obtained.

A papaw of the type already used was fitted with a sampling tube and enclosed in a gas-tight container; the latter was then irrigated constantly with N<sub>2</sub> throughout the experiment. Sticks of potassium hydroxide and a vessel containing pyrogallol were also introduced into the container to assist in the removal of CO<sub>2</sub> and O<sub>2</sub> respectively. Internal and external O<sub>2</sub> concentrations quickly reached zero values, while CO<sub>2</sub> in the container was kept down to a concentration of 0.5 per cent. or less. Immediately after

the commencement of irrigation with  $N_2$ , a quick succession of determinations of internal concentrations of  $CO_2$  and  $O_2$  were made. Thereafter, with continued irrigation, analyses of internal and external gases were undertaken at intervals with the results shown in Fig. 11.

The data demonstrate clearly that transference from air to an atmosphere of almost pure nitrogen is marked by a decrease in the internal concentration of  $CO_2$  to a value which finally is approximately one-half that occurring in the fruit when in air. Taking into consideration the small concentration of  $CO_2$  in the gas-container (approximately 0.5 per cent. throughout the experiment), the corrected internal concentration of  $CO_2$  would have been that indicated by the discontinuous line.

When air was readmitted to the gas-container, a readjustment of internal gas concentration took place, as in the previous experiments, the internal  $CO_2$  concentration finally increasing to the normal value for a ripening fruit.

## VI. DISCUSSION.

In a series of studies Blackman and Parija (1) have considered at length changes in the rate of respiration in apples when fruits were transferred from air to an atmosphere of pure nitrogen. Typical curves obtained by them show that the first effect of placing a fruit in nitrogen is to bring about a rapid increase in the respiration rate, so that the curve rises well above the level of that for normal respiration in air; this is followed by a continuous slow decline. Different classes of fruit have yielded different types of curves; in some the curve for respiration in nitrogen, though declining after the initial sharp rise, has not been observed to return to the 'air-line' though its trend is in that direction; in others the nitrogen-respiration curve eventually falls below the 'air-line', suggesting that metabolic processes, as indicated by superficial liberation of  $CO_2$ , are proceeding at a subnormal rate.

The sharp rise in 'respiration rate' on transference to an atmosphere of nitrogen is accepted by Blackman and Parija as an indication of accelerated metabolism, and accordingly forms an essential part of the elaborate scheme of catalysed reactions by which they interpret the several aspects of apple respiration. From the data submitted in this paper, it is evident that this view requires modification: the high 'respiration rates' recorded when a fruit is transferred from air to nitrogen are not due to accelerated metabolism but are transitional values referable to the amount of  $CO_2$  normally retained in the fleshy tissues, intercellular air spaces and cavity, the maturity of the fruit being important in determining the magnitude of such values. From the information supplied by Wardlaw and Leonard (5) in an earlier paper it may be inferred that transition values for pre-climacteric fruits will be considerably lower than those for post-climacteric because of

differences in the internal concentrations of  $\text{CO}_2$ ; it may further be suggested that the duration of transition effects in different fruits will be dependent on the variable resistance to the diffusion of gases of the flesh at different stages of maturity and to the experimental procedure adopted.

In the present studies on papaw it has also been demonstrated that on removing a fruit from air to nitrogen metabolic processes continue at a rate considerably lower than that observed in air. This is seen to be true both in respect of the rate at which  $\text{CO}_2$  is liberated externally and of the internal  $\text{CO}_2$  concentration which ultimately, in conjunction with tissue resistance, is the factor controlling 'respiration rate'.

It is worthy of note that although Blackman and Parija did not assign to the  $\text{CO}_2$  content of the apple the importance accorded to that factor by the present writer, yet they did not omit it in considering the explanation of phenomena observed on removing a fruit from air to nitrogen. The following quotation (1) makes their position clear:

'The third transitional effect which comes in to mask the combination of the two just described is a purely physical affair, due to the long time it takes to equilibrate the  $\text{CO}_2$  content of an apple with that of its environment by diffusion across the surface, which offers a considerable resistance. If the internal production of  $\text{CO}_2$  were cut off suddenly and completely, the store of  $\text{CO}_2$  in the watery tissues would go on escaping, giving a geometrically decreasing curve of  $\text{CO}_2$  escape to the exterior, and it would be very many hours before this had all escaped to the air current. During all this time there would be an appearance of decreasing  $\text{CO}_2$  production by the apple. The same form of  $\text{CO}_2$  escape, though in a rising sequence, would accompany any instantaneous increase in actual metabolic production, making the rise appear slower than reality, and thus there is always an external distortion of the true form of the change of rate of internal  $\text{CO}_2$  production. It follows that the sudden drop in the  $\text{CO}_2$  production rate, due to the change from nitrogen to air, reveals itself only as a declining rate of escape of moderate duration, taking at least ten hours, and often more, in an imporous apple till the new lower rate of actual production is attained.'

Working on the tomato, Gustafson (3) showed that on transferring fruits from air to nitrogen there was an immediate rise in the rate of liberation of  $\text{CO}_2$  followed by a decline to a steady rate lower than that in air. Replacement of nitrogen by air again brought about a marked increase in  $\text{CO}_2$  output. An interesting feature of his work is that he also replaced air by an atmosphere of pure hydrogen; in these experiments the  $\text{CO}_2$  output immediately rose to very high values, exceeding those obtained on transference from air to nitrogen.

In the absence of oxygen and where a simple carbohydrate is the substrate for respiration, theoretically only one-third of the carbon is used in

CO<sub>2</sub> formation, whereas in air all of it is used. Accordingly Gustafson points out that if the production of CO<sub>2</sub> were the same in air as in nitrogen, three times as much carbohydrate would be used in the latter as in the former. The outstanding fact, however, is that there 'is an increase in CO<sub>2</sub> production when oxygen is excluded'. 'One explanation that comes to mind is that this increase is only apparent, and results only from an outpouring of CO<sub>2</sub> already in the fruit in the intercellular spaces, due to the difference of diffusion of oxygen and nitrogen'. In the absence of information on the internal gas concentrations in the tomato, however, he was unable to substantiate this point of view, but remarks that 'hydrogen, on the other hand, does seem to bring about an outpouring of CO<sub>2</sub>'. He also considers that the more rapid diffusion of hydrogen inwards than that of the fruit gases outwards would probably result in an increase in gas pressure within the fruit. 'Flow of the mixture of gases within the fruit to the outside through the stem scar would take place until equilibrium was attained. This view is further strengthened by the fact that when hydrogen was replaced by air the increase was less than when nitrogen was used. Here the hydrogen diffused out more rapidly than air came in and some CO<sub>2</sub> remained in the intercellular spaces because of a reduction in total pressure.'

These views have been recapitulated in detail, not because they are accepted by the writer, but because they represent an earlier attempt to explain transition effects in terms of physics rather than to regard them as an index of metabolism.

Gustafson further discusses the Blackman scheme of aerobic and anaerobic respiration and concludes that, after the transition period is past, respiration in nitrogen is 'a well balanced or regulated process which merely produces less CO<sub>2</sub> than when fruits are in air'—an opinion supported by the present studies.

Others interested in the movement of gases in and out of fruit by diffusion have also attributed the sharply defined transition effects to causes other than metabolism. Thus Smith (4) has postulated, in terms of gaseous diffusion, that if hydrogen were used in place of nitrogen as the inert gas in anaerobic studies transition effects of a different magnitude would be obtained. This contention, in fact, was proved for apples. The following quotation makes his position clear: 'The transitional effects on the output of carbon dioxide when a change is made from air to hydrogen are, as might be expected, quite different from those which take place in changing from air to nitrogen; and whether or not the latter effects are truly physiological the former are clearly due for the most part to the expulsion of carbon dioxide from the tissues of the fruit. Whether the subsequent history of the carbon-dioxide output is different in hydrogen and nitrogen is a matter for further study. If only physical effects are involved, the difference in the curves may be used to estimate the amount of gaseous carbon dioxide

inside the fruit. If, through a change of acidity, or of the concentration of volatile products, the anaerobic metabolism itself is affected, the method may have a more interesting application.'

## VII. SUMMARY.

1. Consideration is given to the relation between the internal concentrations of  $\text{CO}_2$  and  $\text{O}_2$  in fruits and changes in the gaseous atmosphere by which they are surrounded. For this purpose the papaw was used.

2. The experimental procedure adopted indicates the importance of gradients of gaseous concentration in determining the 'rate of respiration', i.e. liberation of  $\text{CO}_2$  at the surface of the fruit.

3. It is shown that under certain experimental conditions the 'rate of respiration' may bear no direct relation to the rate at which cellular respiration is proceeding at that time.

4. On removing a fruit from one gaseous medium to another, readjustment of the several gas concentrations takes place with great rapidity.

5. The amount of  $\text{CO}_2$  present in the flesh of fruits is of profound importance in relation to measurements of respiration.

6. It has been demonstrated (i) that the high initial 'respiration rates' obtained on removal of fruit from air to nitrogen are purely transitional effects, referable to the amount of  $\text{CO}_2$  present in the flesh and cavity, and (ii) that subsequently metabolic processes continue at a rate considerably lower than that observed in air.

7. These several findings are discussed in relation to those obtained by other investigators.

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## NOTE.

**THE USE OF AGAR IN EMBEDDING SMALL OR SLENDER OBJECTS.**—Although there are times when mycologists must of necessity fix and embed fungi together with the agar on which they are cultured, it is believed that cytologists do not realize the value of agar in embedding.

Agar can be conveniently used to orientate objects for cutting and to facilitate the handling and embedding of very small objects. The agar may be used either before or after fixation :

1. The material can be introduced into the agar while fresh, and fixed immediately the agar sets. This method is convenient for the handling of small objects, and has been used successfully with pollen grains and desmids.

There is, however, a danger that the melted agar may injure delicate objects.

2. The material is fixed, washed, and passed up the alcohols, and *then* transferred to the agar from 70 per cent. alcohol. It is found in practice that the material can be introduced into the agar more easily at this stage than immediately after washing it. This method has been found the more successful. It has proved invaluable in embedding pieces of the long style of *Hedychium* so that they can be kept in order and cut longitudinally: in embedding small and delicate root tips so that they are prevented from curling and can be cut transversely: in embedding filamentous algae so that they can be cut in any plane at will.

A 3 per cent. solution of agar, previously prepared and kept melted, is poured on to a warm slide to form a thin film about one-tenth of an inch thick. The objects to be embedded are introduced into this film as quickly as possible and orientated in some convenient way. After the agar has set, the film is cut so that a neat rectangular block is obtained containing the objects to be embedded. These pieces of agar are quite easily handled and can now be treated just as if they were the objects themselves, to be fixed and washed and embedded in method 1, and embedded in method 2. The agar makes no difference to the technique of embedding provided that chloroform and not xylene is used for clearing and infiltration. Before microtoming, the blocks of wax are trimmed so that the agar has a small portion of wax all round it. It then cuts very easily. The agar, of course, remains on the slide after the wax is melted away, but makes no difference to the subsequent treatment. It has the advantage of helping to fix small objects to the slide during the process of staining. The agar looks quite transparent, but stains faintly especially with acidic dyes.

I am indebted to Mr. Horton, of Liverpool, for a suggestion that agar might be of use in this connexion.

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# Further Studies on Transport in the Cotton Plant.

## VI. Interchange Between the Tissues of the Corolla.<sup>1</sup>

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With six Figures in the Text.

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### I. INTRODUCTION.

SCHUMACHER, 1931 (12), has shown that the sudden wilting of many flowers is the outward manifestation of a preceding intense hydrolysis of protein and that as much as two-thirds of the nitrogen present may be exported from the corolla within a period of twenty-four hours. He observed that this rapid movement of nitrogen takes place for the most part from wilted tissues. Ball, 1933 (1), studied the changes in fresh and dry weights of the flowers of *Turnera elegans* before, during, and after flowering. Both fresh and dry weights rose fairly steadily until the flowers opened and

<sup>1</sup> Paper No. 16 from the Physiological Department of the Cotton Research Station, Trinidad.

[*Annals of Botany*, Vol. L. No. CC. October, 1936.]



then declined. He concluded that about 60 per cent. of the dry material of the perianth passes back into the plant before the flower is shed. Combes, 1935 (3), has shown that minerals are exported from the corolla of *Lilium croceum* during the progressive wilting that precedes death.

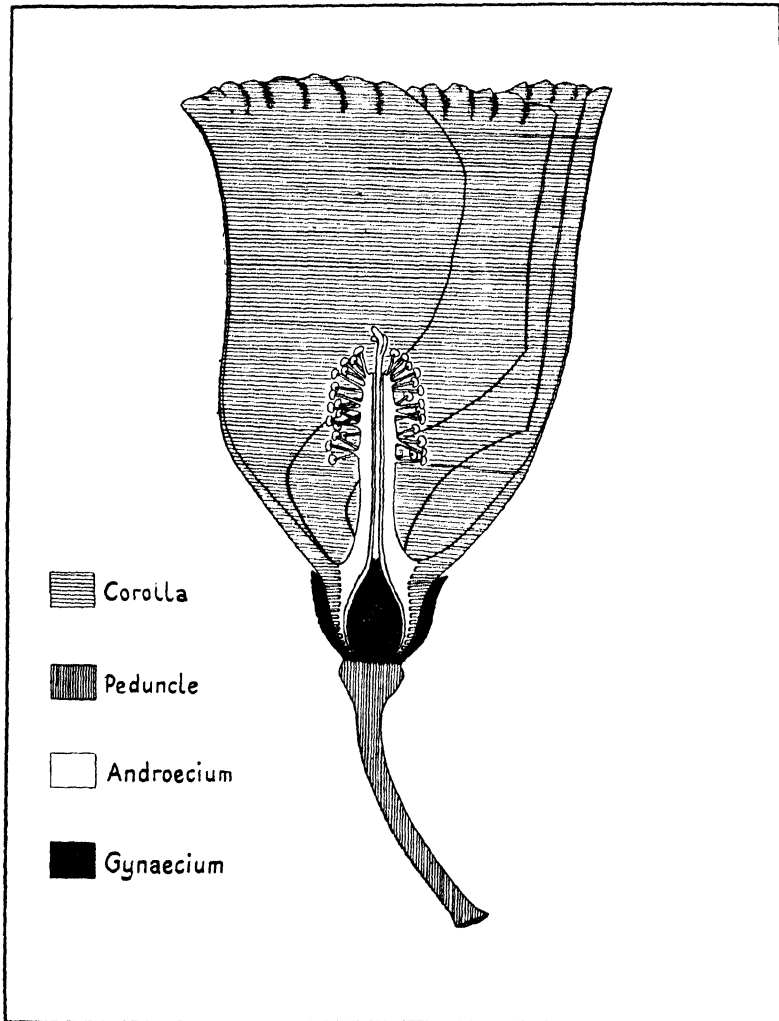


FIG. 1. Diagram showing method of subdividing flower.

It would thus appear that the *Rückgang* of materials so characteristic of the ageing foliage leaf is also a feature of floral leaves. There is, however, a distinction in that the *Rückgang* process is enormously speeded up in the floral leaf. In a recent paper (10) we suggested that the export of sugar from the foliage leaf was in a large measure conditioned by the *polar*

activity of the transition cells in the bundle ends. It was considered probable that the transition cell might also play a part in promoting the export of mineral elements. As the leaf, whether floral or foliage, must, in its youth, import much of its food materials through the phloem, it seems clear that the polarizing mechanism in the transition cell cannot be as active in early as in late life. In the present paper, we have attempted to unravel some of the factors which are responsible for the change in the direction of movement of food materials that occurs during the life of the corolla.

## II. METHODS.

### (A) *Material.*

The flowers of Sea Island Cotton in Trinidad begin to open in the morning about 8 o'clock and the petals begin to wilt and redden basipetally towards the evening. Abscission of the corolla and the attached androecium occurs on the following day. The withered style and stigma are shed with the staminal column (cf. Fig. 1). In separating the various parts of the flower for analysis, the following procedure was adopted. Before the experiment began, the leafy involucre was removed. It is not, therefore, shown in the diagram. To collect the flower, the peduncle was broken away from the fruiting branch. In the laboratory the corolla and the attached staminal tube were removed from the receptacle. The androecium was then torn away from the corolla. The ovary and calyx with a film of the receptacle were then separated by cutting from the peduncle. The flower was thus separated into four regions, which for convenience will be referred to as the *corolla*, the *androecium*, the *gynaecium*, and the *peduncle*.

The yellow petals contain a thin, loosely arranged mesophyll about three cells thick, which becomes thicker and more compact at the proximal end. The stomata are very sparsely distributed and the guard cells contain starch. The fine veins are similar to those of the foliage leaf (cf. 10) and are enclosed in a sheath of a single layer of border parenchyma. The transition cells are well developed.

### (B) *Analysis.*

The following determinations were made by methods previously described:

*On oven-dried material:* Total nitrogen (6): phosphorus, potassium, calcium (8): magnesium, chlorine (7).

*On alcohol extracts:* Sugars (9).

*On expressed sap:* Crystalloid nitrogen (6): Total phosphorus, phosphate phosphorus, total potassium, total calcium, total magnesium, and total chlorine (9).

Values for bound water and the weights of sap soluble materials corrected for bound water (9) were calculated from the foregoing determinations. Results are expressed on the sample basis and represent the weights of material in one hundred flowers, &c. Concentrations are expressed as gm. per 100 gm. water.

### III. CHANGES DURING DEVELOPMENT (EXPERIMENT 1).

#### (A) *Procedure.*

The flowers were collected from a population producing about six thousand flowers per day. Ten collections were made. Each collection consisted of two samples, and each sample contained two hundred flowers. The initial collection was made on the day before the flowers opened.

#### *Time-table.*

Jan. 14 1935	Initial collection (5p.m.).
„ 15 „	1st collection (6 a.m.).
„ „ „	Involucres removed (6.30–8 a.m.).
„ „ „	2nd collection (8 a.m.).
„ „ „	Flowers opening (8–9 a.m.).
„ „ „	3rd collection (10 a.m.).
„ „ „	4th collection (noon).
„ „ „	5th collection (2 p.m.).
„ „ „	6th collection (4 p.m.).
„ „ „	Tips of corollas begin to redden (4–6 p.m.).
„ „ „	7th collection (6 p.m.).
„ 16 „	8th collection (8 a.m.).
„ „ „	9th collection (10 a.m.).

In the laboratory the flowers were subdivided into the four regions already described, viz. corolla, androecium, gynaecium, and peduncle. Full analyses were made only on the corolla. In the three other regions, only the dry weight, weight of water, and the total amounts of the mineral elements were estimated. In addition, a number of corollas were collected throughout the course of the experiment for observations on the distribution of starch and peroxidase.

#### (B) *Results.*

(1) *Movement and concentrations in corolla.* The continuous lines in Fig. 2 show the changes in the weights of nitrogen, potassium, phosphorus, &c., in the corolla throughout its life period. The broken lines show the concentrations of these elements in the expressed sap. In both cases the results are expressed as percentages of the maximum values. The night

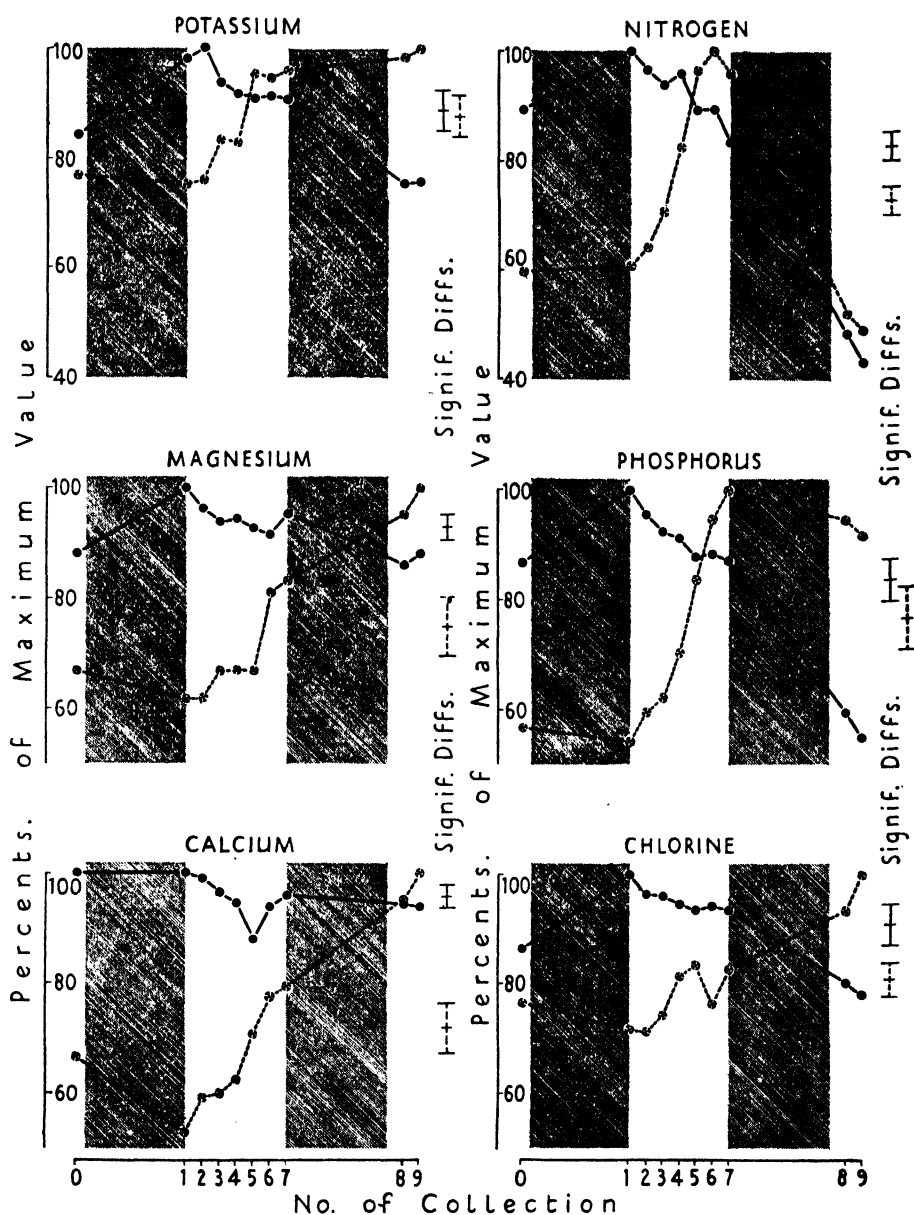


FIG. 2. Changes in the total amounts (continuous lines) and in sap concentrations (broken lines) of mineral elements in the corolla. All values expressed as percentages of the maximum. Significant differences ( $P = 0.05$ ) are shown on the right of the graphs (Experiment 1).

periods between 6 p.m. and 6 a.m. are shaded in the graph. Significant differences ( $P = 0.05$ ) are shown on the right of the graphs.

Taking first the changes in the amounts of the various elements, it will be seen that, with the exception of calcium, materials entered the corolla during the night prior to anthesis. As the increase in dry weight per hundred corollas between collections 0 and 1 was 4.09 gm. while the increase in water was 35.08 gm., it would follow that if import into the corolla had occurred in the xylem, the concentration of the solution entering would have been 11.6 gm. dry material per 100 gm. water. The concentration of dry material in the transpiration current (cf. 8) is, however, of the order of 0.120 gm. per 100 gm. water or about 1/100 of that calculated from the weights of dry material and of water that actually entered the corolla. It will be clear that either entry occurred via the phloem and not the wood, or else that there was a great<sup>1</sup> loss of water from the corolla during the night. The latter possibility may be dismissed as (1) the unopened corolla exposes a relatively small surface, (2) the surface was coated with dew during the night, and (3) stomata are very sparsely distributed. Similar considerations obtain for nitrogen, potassium, phosphorus, &c. It will be observed that calcium, which previous work has shown to be immobile in the phloem, did not enter the corolla during this period. It may be concluded that phloem transport was active prior to the opening of the flower and that movement at this time was in the direction of the corolla.

During the ensuing day and night (i.e. between collections 1 and 9) there was a loss of all materials, including calcium, an element which we have grounds for believing is not mobile in the phloem. The loss was greatest for nitrogen (56 per cent.) and phosphorus (45 per cent.), and least for magnesium (12 per cent.) and calcium (6 per cent.). The losses of potassium (24 per cent.) and chlorine (22 per cent.) were intermediate. The changes in moisture are shown in Fig. 3. It will be noticed that the rate at which water was lost was more rapid by day than by night.

Reverting to the changes in the amounts of the mineral elements, the losses of nitrogen and phosphorus were greater by night than by day, while for calcium the loss during the day was followed by an increase during the evening and no significant loss during the ensuing night. The behaviour of magnesium during the day, and to a less extent potassium and chlorine, paralleled that of calcium. This loss and gain of calcium during the day suggests that it may have been sucked back with water into the body of the plant, while transpiration from the foliage leaves was active and returned to the corolla when the water deficit was reduced in the evening. The absence of a significant loss of this element, which is immobile in the phloem, during the

<sup>1</sup> The weight of water in a single corolla at collection 1 was 1.05 gm. and the weight that would have to be transpired between collections 0 and 1 to account for the uptake of dry materials would be about 35 gm.

night, while the loss of nitrogen, phosphorus, potassium, &c., was greatest, tends to reinforce this view. The losses of the other elements during the day may also be wholly or partially due to transport via the wood. It seems probable, however, that loss through the phloem did not become very active

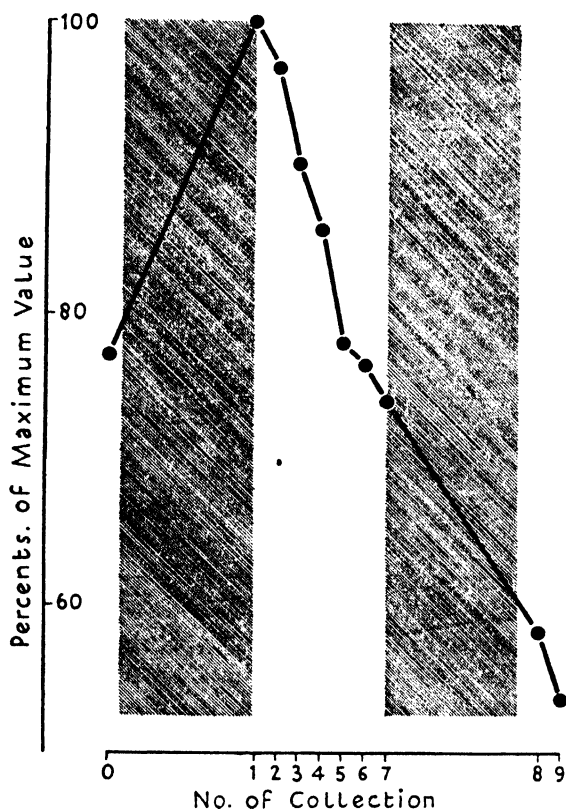


FIG. 3. Changes in the amounts of water in the corolla. Values expressed as percentages of the maximum (Experiment 1).

until the evening. However this may be, there must have been a reversal in the direction of phloem transport between the first and second nights; materials entered the corolla via the phloem during the first night and left during the second.

It will be seen that the *concentrations* remained low during the night prior to anthesis and that they rose during the following day. The increases between collection 1 and the evening collections in the concentrations of nitrogen and phosphorus were considerable and were much greater than for the other elements. The *maximum* percentage increases between the morning collections and the evening collections were nitrogen 40, phosphorus 46, magnesium 23, calcium 26, and chlorine 12. In the case of chlorine the increase was entirely due to a loss of water; the export of chlorine was

in fact small relative to the loss of water. For the other elements, the conversion of insoluble to soluble materials also obtruded itself as a factor.

If gradient controls transport, it would thus appear that the change in the direction of movement might be due to a change in concentration in the corolla, for the concentrations in the body of the plant may be assumed to have remained constant. Export via the phloem does not appear to have been rapid until after a considerable rise in concentration. There are, however, reasons which suggest that there are other factors at work in causing the reversal of movement. In the first place, the concentration of crystalloid nitrogen fell on the second night to a lower level than on the first night, while import was proceeding, without any effect on the rate of export. In this case, crystalloid nitrogen may not be a satisfactory indicator of the mobile compound. Furthermore, although the rise in chlorine concentration was due only to a loss of water, which should affect the transport just as a change in concentration brought about by chemical or physical transformation, it is not a wholly convincing cause for the change in the direction of chlorine transport.

*Movement in whole flower.* Our object now is to ascertain the destination of the materials exported from the corolla during the second night, while export from the corolla appears to have been limited to the phloem. The changes in the amounts of the various elements in the flower are expressed graphically in Fig. 4. The scales for the whole flower are shown in the centre, while the scales for the component parts into which the flower was subdivided are situated on the margins of the graph. It will be observed that the scales are the same, but the origins for the whole flower differ from those for the component parts.

For the whole flower the results are very similar to those for the corolla. Gains of varying extent were registered during the first night. These were followed by losses, presumably via the wood, during the morning and re-entry during the evening. Fully significant<sup>1</sup> losses are shown during the second night in nitrogen, phosphorus, potassium, chlorine, and magnesium. For calcium there is no evidence of phloem transport. The loss and gain during the previous day are partially or wholly via the wood. It will be clear that the phloem mobile elements that were exported from the corolla cannot have remained in the other parts of the flower, but must have travelled down the peduncle and entered the fruiting branch. In the next section, we will, therefore, compare the sap concentrations of the various elements in the corolla and in the bark of the parent branch during the period of active export through the phloem. Before doing so, however, it will be of interest to review the changes that occurred in the component parts of the flower.

The changes in the corolla have already been discussed. Reference to

<sup>1</sup> Significant differences are not shown on the graph.

the graph will make it clear that it was responsible for the bulk of the nitrogen (84 per cent.), phosphorus (97 per cent.), and more than responsible for all the potassium (109 per cent.) that left the whole flower and travelled

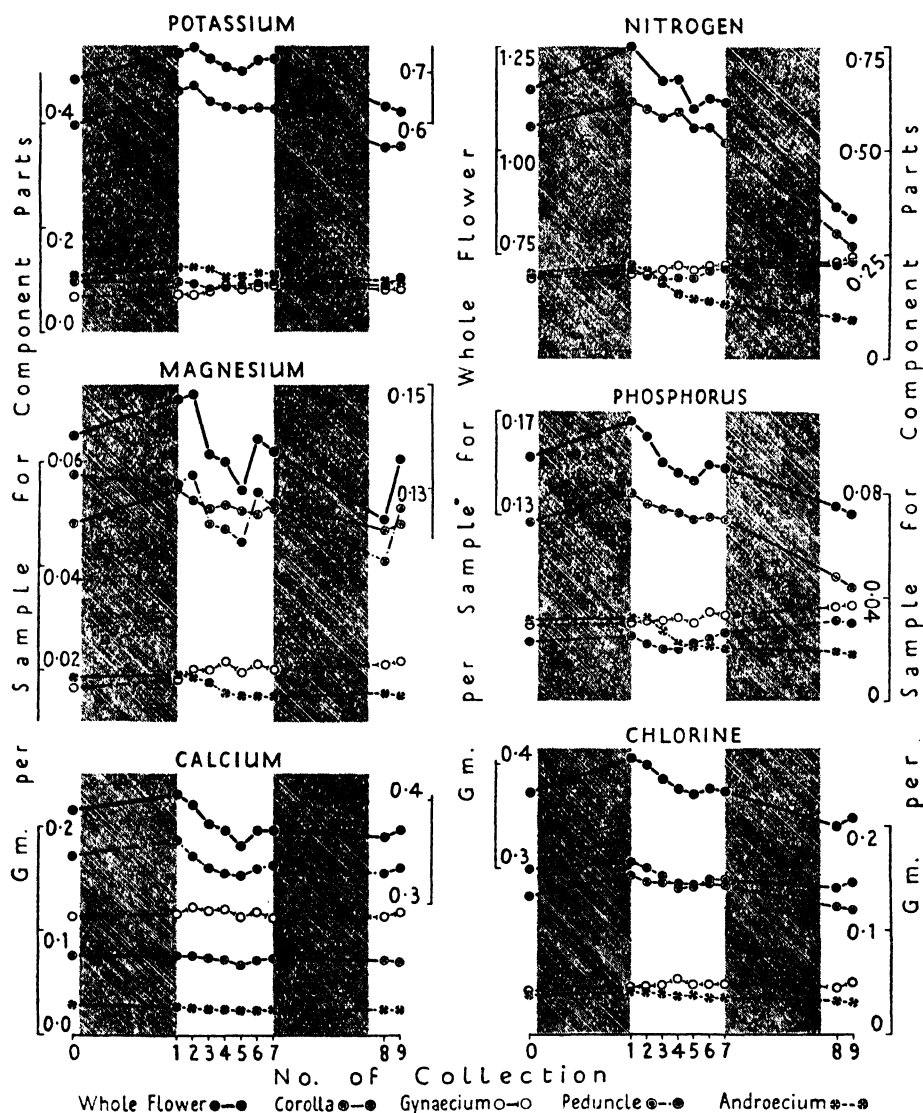


FIG. 4. Changes in the total amounts of mineral elements in the whole flower and in its component parts. Values expressed in gm. per one hundred flowers (Experiment 1).

back into the fruiting branch. It also accounted for 58 per cent. of the chlorine and 52 per cent. of the magnesium exported from the whole flower. The behaviour of the androecium paralleled that of the corolla, but the losses were more marked during the day than the night. The



peduncle is chiefly interesting for the marked losses during the morning and the re-entry of elements during the evening. There were net losses of calcium, magnesium, and chlorine between the first and the final collections. Nitrogen and phosphorus on the other hand registered gains.

#### IV. CONCENTRATIONS IN COROLLA AND FRUITING BRANCH

(EXPERIMENT 2).

##### (A) *Procedure.*

The procedure in this experiment differed from that in the previous experiment in three respects. Firstly, samples were not collected on the day prior to anthesis; the initial collection was made in the morning just as the corollas opened. Secondly, the only part of the flower sampled was the corolla, the fleshy basal part being rejected with the staminal tube. Thirdly, the internodes on either side of the node to which the peduncle was attached were collected and the sap concentration in the bark determined.

##### *Time-table.*

Feb. 21.	1933.	Corollas opening.	Initial collection of corollas (7-8 a.m.).
"	"	"	1st collection of corollas and of fruiting branches (10 a.m.).
"	"	"	2nd collection of corollas and of fruiting branches (2 p.m.).
"	"	"	3rd collection of corollas and of fruiting branches (6 p.m.).
"	22	"	4th collection of corollas and of fruiting branches (8.30 a.m.).
"	"	"	5th collection of corollas and of fruiting branches (12.30 p.m.).

##### (B) *Results.*

The weights of the various materials in the corolla expressed as percentages of the maximum values, as well as the sap concentrations in corolla and bark of fruiting branch, are shown in Fig. 5. As materials exported from the corollas must have entered the bark of the fruiting branch, it becomes of interest to find out whether movement was with or against a gradient. For nitrogen, there was, as in the last experiment, a considerable export from the corolla, and this export was presumably through the phloem. The concentration of crystalloid nitrogen in the corolla was on the average in excess of that in the fruiting branch, but during the night the concentration in the corolla fell below that in the fruiting branch. The results for nitrogen are not, therefore, suggestive of movement in response to gradient. As we have already indicated,

however, crystalloid nitrogen may be an unsatisfactory indicator of the mobile form of nitrogen. For phosphorus, the concentrations of total sap

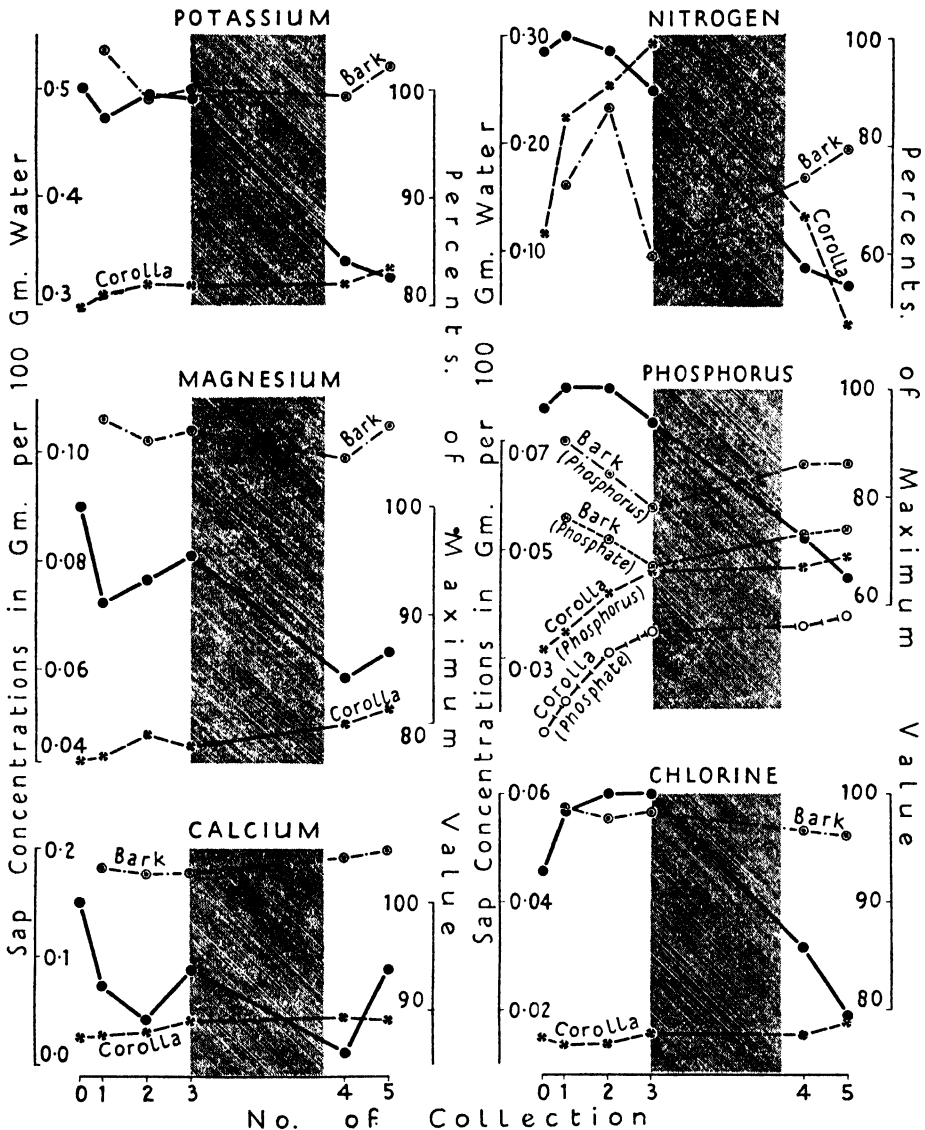


FIG. 5. Changes in the total amounts (continuous lines) of mineral elements in the corolla and changes in sap concentrations (broken lines) in the corolla and in the fruiting branch bark. Total amounts expressed as percentages of the maximum and concentrations in gm. per 100 gm. water (Experiment 2).

soluble phosphorus and of phosphate phosphorus are recorded. For both, the concentrations in the fruiting branch exceeded those in the corolla. For non-phosphate phosphorus, the concentrations are about

the same, being slightly greater in the fruiting branch. Nothing very decisive, therefore, emerges from a consideration of phosphorus gradients.

For chlorine, however, the concentrations are about five times as great in the fruiting branch as they are in the corolla. As all the chlorine appears to be in solution and to be ionizable (cf. 9), the results suggest that it has moved out of the corolla and into the fruiting branch against a steep gradient. Movement down a gradient is not, however, excluded, for we know nothing of the regional differences in chlorine concentrations in either the corolla or the bark. For potassium and magnesium, also, movement appears to have proceeded against the gradient. For these elements, and for calcium, there was the same exit of material during the morning and re-entry during the evening, as we observed in the previous experiment.

#### V. DISCUSSION.

It will be evident that our attempts to explain the reversal of the direction of transport in the corolla in terms of gradient have not been successful. Consideration of the changes in concentration in the corolla shows that phosphorus, potassium, magnesium, and chlorine were imported prior to anthesis, while the concentrations were relatively low and were exported after the rise in concentration that occurred during the day the flowers opened. For phosphorus, potassium, and magnesium the rise in concentration arose partly from a conversion of insoluble into soluble forms (cf. Fig. 6) and partly from a drying out of the tissues. For chlorine, the rise in concentration was due only to the drying out of the tissues. For nitrogen, the concentration of crystalloid (non-protein) nitrogen diminished greatly without any effect on the rate of export. The concentrations in the corolla and the bark of the parent fruiting branch render it probable that export from corolla to fruiting branch proceeded against a gradient. The evidence that transport proceeded against a gradient is best for chlorine; there can be little doubt that we are, for this element, actually dealing with the mobile form.

It will have been observed, perhaps, that results for carbohydrate have not been presented. The reason for this omission is that we were unable to measure the loss that occurred from the corolla as a result of respiration. The loss in dry weight from corollas removed from the plant was, however, less than half of that from corollas on the plant. If we accept this difference as being due to export, the behaviour of carbohydrate would fall into line with that of other materials, for the sucrose concentration was about five times greater in the bark than in the corolla. Sucrose is assumed to be the mobile form of carbohydrate out of the corolla by analogy with the foliage leaf (cf. 10).<sup>1</sup> Although regional analyses of the corolla and fruiting branch

<sup>1</sup> It is of interest to note that the polyglucoside characteristic of the foliage leaf is also present in the corolla (cf. 10).

were not carried out, there can be little doubt that the reversal of direction of transport between fruiting branch and corolla was not solely determined by gradient considerations.

The anatomy of the veins in the petal is very similar to that of those

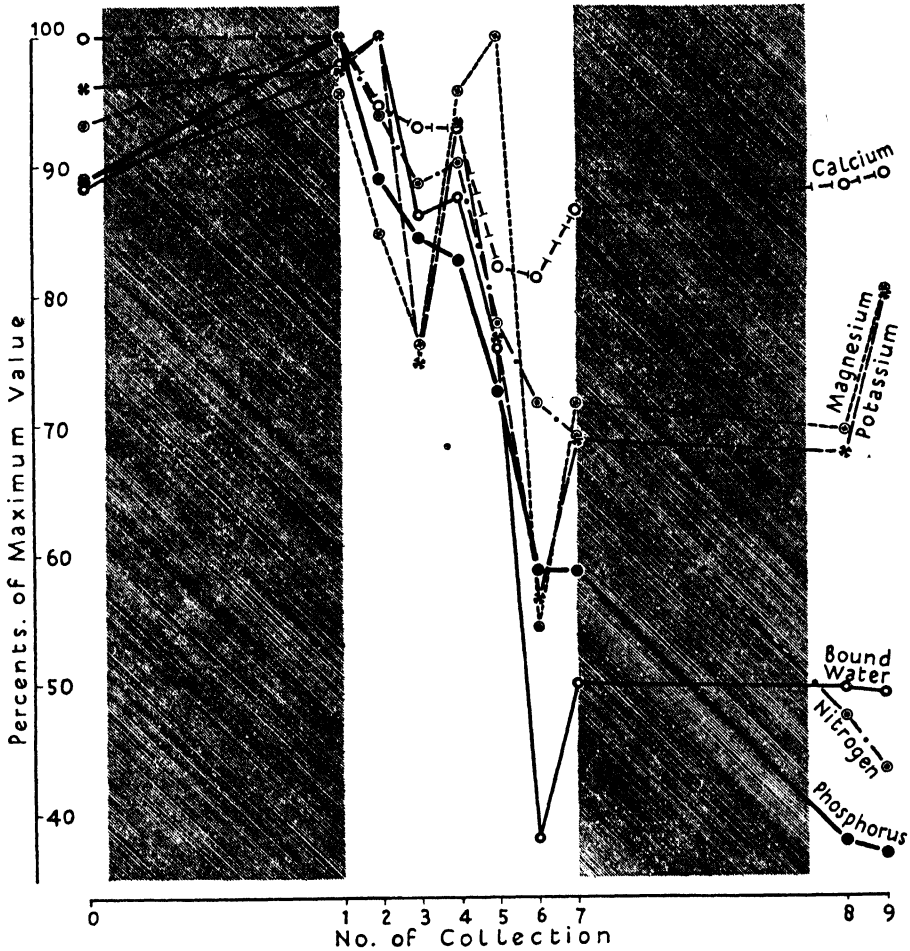


FIG. 6. Changes in bound water and in the insoluble fractions of the mineral elements in the corolla. Values expressed as percentages of the maximum (Experiment 1).

in the foliage leaf. The chief characteristic of the fine veins is the presence of companion cells (transition cells) which are much larger than the sieve-tubes. We suggested (10) that in the foliage leaf the function of these cells is that of accumulation of solutes from the mesophyll with liberation of the accumulated solutes into the sieve-tube for longitudinal transport. Horizontal movement across the transition cell was conceived of as being polarized, and movement from the mesophyll to the transition cell as

occurring either with or against a gradient depending on the rates of production in the mesophyll and of consumption in the body of the plant, energy relations of course being involved. While a mechanism of this type would account for the export of materials against a gradient in the corolla, it is necessary to assume that the accumulating mechanism is inactive during the period of corolla expansion while import was proceeding. As the veins begin to show a strong peroxidase reaction (cf. Keeble and Armstrong (4)), just about the time that export begins, there would appear to be some grounds for this view. *Microscopic* examination of the distribution of the peroxidase (cf. 11) in the veins shows, however, that it is located in the transition cells and that it is present even during the period of corolla expansion, but that it is masked by starch in the sheath at this time and only becomes *macroscopically* visible after the disappearance of the starch,<sup>1</sup> which occurs about the time that export becomes active.

We have already alluded to the *reddening* and *wilting* of the petal in the evening of the day of anthesis. The colour change from yellow to red spreads gradually from the apex to the base of the petal. The veins, however, remain yellow and turgid long after the *parenchyma*<sup>2</sup> between the veins has reddened and wilted. It seems probable that the reversal of the direction of transport is associated with some change in the parenchyma rather than in the phloem. As the latter is in direct communication with the phloem in the body of the plant and undergoes no visible change, it seems probable that the concentration of solutes in it remains unaltered during the development and senescence of the parenchyma.

The rapidity with which insoluble materials were transformed into soluble forms in the corolla is shown in Fig. 6. The values recorded in the graph represent the weights of insoluble materials in the corollas in Experiment 1 and are expressed as percentages of the maximum amounts. It seems probable that these changes were limited to a large extent, if not wholly, to the parenchyma between the veins. Separation of the petals into veins and parenchyma has not proved practical, and so we have not been able to test this probability experimentally. For nitrogen and phosphorus the maximum amount of insoluble material was present at collection 1, after which there was a rapid conversion of more than 50 per cent. of the insoluble fractions into sap soluble forms. For magnesium and potassium about 30 per cent. of the insoluble material was converted into soluble forms, while for calcium only about 15 per cent. was so converted. It is of considerable interest to find that while these changes

<sup>1</sup> On the day prior to anthesis both vein and parenchyma contain much starch, which, however, disappears from the parenchyma except the guard cells before the flower opens. This difference in the time of disappearance of starch supports the visual observations on the relative stability of parenchyma and vein.

<sup>2</sup> In an earlier paper we referred to the parenchyma between the veins of the foliage leaf as the mesophyll (cf. 10).

were occurring, there was also a rapid conversion of bound<sup>1</sup> into free water. It would seem as if the protoplasmic organization collapsed with great rapidity. In this connexion it is of interest to note that Lepeschkin (5) concluded that the short life of the corolla of *Cichorium Intybus* is due to a particularly great instability of the principal compounds of its protoplasm. It would appear that the cause of the reversal in the direction of transport must be sought in these very rapid changes in the parenchyma and not to any alteration in the activity of the transition cell. This suggestion is supported by Schumacher's (12) observation that the export of nitrogen takes place only from the corollas of plants in which protein hydrolysis and wilting of the corolla occurs.

It has been hitherto assumed that in the interchange of solutes between the phloem on the one hand and the supplying and receiving tissues and organs on the other, the latter play a purely passive role. The only cells conceived of as being capable of actively accumulating solutes against a gradient have been the companion and transition cells. Apart from the activity of these cells, interchange was considered to be determined solely by gradient considerations. Thus, sugar was pictured as moving to the developing seed from the sieve-tube as a result of the sucrose gradient; a low concentration of sucrose being maintained in the developing seed as a result of the rapid utilization (transformation) of this sugar. The changes taking place during the ripening of the corolla suggest, however, that the tissues external to the phloem (i.e. the parenchyma or mesophyll) may play an active part in determining the direction of solute movement. Steward's (14) most interesting work on accumulation by storage organs and by leaves reinforces this suggestion.

A clue to the type of mechanism at work is suggested by some of the experiments reported in our work on 'The Polar Distribution of Sugars in the Foliage Leaf' (10). Thus, in Experiment 6, the mesophyll with a mean (for four days) concentration of 0.41 gm. sucrose per 100 ml. sap showed a diurnal increase of 242 per cent., while the fine veins with a mean calculated concentration of 0.67 gm. per 100 ml. sap showed a diurnal increase of 260 per cent. Thus, although movement took place from a region of low to one of high concentration, and although the actual diurnal rise in concentration was greater in the region of high than in that of low concentration, the *relative* increases were much the same in both regions. In another experiment (Experiment 7) observations were made on the sugar concentrations of the tissues of the leaf as carbohydrate travelled from illuminated to comparable leaves which had been darkened. As the stems below the foliage leaves were ringed the sugar concentration in the foliage region above the ring increased during the experiment. By expressing the equilibrium concentration in each tissue as a percentage of the concentration

<sup>1</sup> Bound water expressed as a percentage of total water ranged from 21 to 7 per cent.

in the inner bark, which included the whole phloem, the following results were obtained :

		Sucrose		Total sugars	
		Illuminated.	Darkened.	Illuminated.	Darkened.
Lamina	Mesophyll	21.1	8.5	39.6	24.4
	Vein	38.9	29.9	56.9	52.4
Petiole	Outer bark	56.7	54.8	58.6	58.7
	Wood	64.1	64.1	77.7	87.7

The relative sucrose concentrations in the petiolar tissues show good agreement in view of the fact that the concentrations in the Illuminated group were about 50 per cent. greater than those in the Darkened group. For the lamina the agreement was not so good. If, however, total sugars are considered instead of sucrose, as seems permissible in view of the possibility of sucrose hydrolysis during the process of separation of vein and mesophyll, the relative percentages in the vein are in good agreement. The divergences in the mesophyll, which are considerable, may well be due to the fact that in one case it is a supplying, while in the other it is a receiving, organ.

These experiments suggest that the *relative* sucrose concentrations of adjacent tissues tend to remain constant under comparable conditions, and are independent of the *actual* concentrations. This is what would happen if sucrose were distributed among the various tissues in accordance with the law governing the distribution of a solute between immiscible solvents, and suggests that the various tissues behave as if they had different solvent capacities for sucrose.

The analogy between accumulation by a cell from an external aqueous medium, as investigated by Steward (14), and from other cells as occurs in the movement of sucrose between the phloem and adjacent tissues, suggests that the mechanism involved may be similar in the two cases. Steward has shown that accumulation from an external medium is dependent on energy supplied by respiration, and, provided that this is maintained, should proceed until the concentration in the external medium is infinitely small (13). He also found that different tissues possess different degrees of accumulating capacity. If, now, we consider the distribution of salt between adjacent cells with differing degrees of accumulating capacity, the equilibrium attained will depend on the type of accumulating mechanism pictured. It might be that the balance of accumulating capacity would determine the direction of movement, in which case movement would proceed towards the cell with the larger accumulating capacity until it had accumulated all the salt. On the other hand, it might be that the salt would be divided between the cells in proportion to their relative accumulating capacities. In the former case, energy might be utilized directly in accumulation, while in the latter case, it might be utilized in

maintaining different solvent capacities in the various cells. It is this latter type of mechanism which would enable results such as we have described for the movement of sucrose between the phloem and adjacent tissues to be obtained. It would also harmonize with the rapid diurnal exchange of sugar between the petiolar cortex and phloem with their widely differing concentration levels (Experiment 4 (10)).

The explanation of movement in the corolla based on the analogy with solubility would be somewhat as follows. Solutes would move between phloem and mesophyll to maintain the distribution determined by the relative solvent capacities of the two tissues. Utilization in the mesophyll as development proceeds would maintain movement from phloem to mesophyll. After anthesis, the cessation of utilization would lead to a cessation of import. With the onset of protoplasmic decomposition and the drying out of the tissues the concentration in the mesophyll would rise and solutes should move back into the phloem. If the solvent capacity of the mesophyll declines with senescence, and the work of Steward suggests that this is so, another factor promoting export from the mesophyll would be introduced.

In a previous paper (10), we suggested that the transition cell accumulated sucrose from the mesophyll and liberated it into the sieve-tube. This transport across the transition cell was described as *polar*. The sieve-tube was not regarded as possessing the power of accumulation. The intimate cytoplasmic connexion between the sieve-tube and transition cell does not, however, indicate that there can be any marked difference in the solvent capacity between the two cells. It seems more probable that both types of cell have the same solvent capacity, but that the sieve-tube is concerned mainly with longitudinal transport and is relatively impermeable except where it is in contact with the transition cell, while the latter is concerned with the exchange of solutes between it and the neighbouring cells. On this view the sieve-tube should be less permeable than the transition and companion cells.<sup>1</sup> One is led to suggest that the solvent capacity of a tissue for a particular solute may be related to the diffusion constant of the solute through it. In the sieve-tube/companion cell complex both are probably greater than in other tissues. As yet, however, we know little or nothing of the conditions that prevail in the other tissues.

## VI. SUMMARY.

1. During the night preceding anthesis, nitrogen, phosphorus, potassium, magnesium, and chlorine are imported into the corolla and during

<sup>1</sup> Crafts (2) found that he could plasmolyse the companion cell but not the sieve-tube. He concluded that the latter was highly permeable, but does not appear to have considered the possibility that the sieve-tube may have been disorganized as a result of the release of pressure that may have occurred in section preparation.



the succeeding night these elements are exported. The bulk of the materials exported travel through the peduncle to the parent fruiting branch. Reasons are given for believing that both import and export take place through the phloem.

2. The sap concentrations remain low while import is proceeding and rise during the day of anthesis prior to export. The rise in concentration is due in part to conversion of insoluble to soluble materials and in part to a drying out of the tissues of the corolla.

3. As materials are exported from the corolla to the fruiting branch, and as the concentrations are much larger in the bark of the fruiting branch than in the corolla, it is inferred that gradient considerations alone are not responsible for the change in the direction of movement in the corolla.

4. The attempt to explain the reversal of the direction of transport in terms of a change in the activity of the transition cells was not successful. As the parenchyma between the veins turns from yellow to red and wilts just before export begins, while the veins remain yellow and turgid, it was suggested that the cause of the reversal of the direction of transport should be sought in the parenchyma between the veins rather than in the vein and its phloem.

5. Re-examination of some of the data presented in a recent paper on carbohydrate transport indicates that the distribution of sugar between tissues conforms to the law governing the partition of a solute between liquids with different solvent capacities.

6. The reversal of the direction of transport in the corolla is explained as follows. Solutes move between the phloem and the parenchyma between the veins to maintain the distribution determined by the relative solvent capacities of the two tissues. Continued movement from the phloem into the parenchyma is ensured during development by the maintenance of low concentrations as a result of utilization and of the intake of growth water. After anthesis the cessation of utilization would lead to a cessation of import. With the onset of protoplasmic decomposition and the drying out of the tissues, the concentration in the parenchyma rises and solutes move back into the phloem. If the solvent capacity of the parenchyma declines with age, another factor promoting export from the parenchyma would be introduced.

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# The Effect of Certain Accessory Growth Substances on the Sporulation of *Melanospora destruens* and of some other Fungi.

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With Plate X and one Figure in the Text.

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## I. INTRODUCTORY.

IN a previous paper (1) an account was given of the effect of certain fungi on the formation of perithecia by *Melanospora destruens* Shear. It was shown that these were formed earlier and in greater numbers in the presence of certain other fungi than in pure culture. The media on which such fungi had grown for some time had a similar stimulatory effect. Experimental evidence was obtained indicating that this effect was due to the presence of some substance or substances produced by the fungi used as staling<sup>1</sup> agents. Fractionation with ether in acid solution showed this substance to be ether-insoluble. This fact suggested a comparison with other growth-regulators which have been described elsewhere, such as Nielsen's 'growth substance B' (14), the 'Bios' substance of Wildiers (20), and a fungal growth-promoting substance investigated by Farries and Bell (7).

<sup>1</sup> Throughout this paper the word 'stale' refers to a medium in which an organism has been grown for some time, without necessarily meaning that the medium has become so 'stale' as to inhibit or seriously to repress growth.

Farries and Bell, working with *Nematospora gossypii* and related fungi, found that an ether-insoluble substance, present as an impurity in certain nitrogenous preparations, viz. commercial peptone, white of egg, and milk casein, was essential for good growth of these organisms. The chemical investigation of this growth regulator was continued by Buston and Pramanik (3) and by Buston and Kasinathan (5), who found that the active principle could readily be obtained in bulk from dried lentils. The latter were steamed, filtered, and the filtrate was precipitated twice with 95 per cent. alcohol, in order to remove the bulk of the protein. The alcoholic filtrate was concentrated to remove most of the alcohol and standardized. The extract thus obtained is referred to as crude lentil extract. Buston and Pramanik were able, by precipitation with barium hydroxide, to separate the active substance into two fractions, each of which was essential for growth of *Nematospora*. The baryta precipitate fraction, or first accessory factor, was identified as *D*-inositol. The filtrate, or second accessory factor, has not yet been identified.

## 2. METHODS.

The experimental methods used, and particularly the method of obtaining comparative values for perithecial frequency, were, in general, similar to those already described in an earlier paper (1).

Substances, the spore promoting activities of which were to be tested, were added to a standard medium (Medium A of the previous paper, consisting of glucose, 5 gm.; potassium nitrate, 3.5 gm.; magnesium sulphate, 0.75 gm.; potassium dihydrogen phosphate, 1.75 gm.; agar, 15 gm.; distilled water, 1 litre) unless otherwise stated. As a rule plates were poured with the test-medium in the usual way, but in some cases this was used to replace a segment of a plate of Medium A.

Where 'staled' media were used, these were prepared by the methods described in detail in the earlier paper. Occasionally it was desirable to avoid the inclusion of staling products and a possible check to growth, so that a mycelial extract of certain fungi was used. The mycelial mat of *Botrytis cinerea* or *Fusarium fructigenum*, which had been grown in Medium A without agar at laboratory temperature for two weeks, was removed, washed, and then boiled in distilled water for thirty minutes. The mycelium was then removed by filtration through fine muslin and the liquid made up to the required strength. These extracts stimulated the formation of perithecia of *M. destruens* to an extent comparable with that produced by the staled media.

It should be noted that, during the course of the work described in the previous paper, the strain of *M. destruens* used gradually spored less freely. When the investigation with which this paper is concerned was begun the

fungus no longer produced its perithecia on Medium A alone. The results obtained are, therefore, all the more striking.

### 3. THE EFFECTS OF A CRUDE LENTIL EXTRACT AND OF FRACTIONS OF THIS EXTRACT ON SPORULATION OF CERTAIN FUNGI.

Samples of the crude lentil extract, of inositol, and of the inositol-free fraction were kindly supplied by Dr. Buston, and their effects on the sporulation of *M. destruens* were tested.

A sufficient quantity of crude lentil extract was added to Medium A to give 0.2 per cent. (dry weight) of the extract in the medium (this being

TABLE I.

Medium.	Perithecial frequency	
	5 days after inoculation.	9 days after inoculation.
Medium A	0	0
A + 0.2 % crude lentil extract	Many, immature	10.2
A + <i>l</i> -inositol	0	0
A + inositol-free fraction	Many, immature	9.03
A + both fractions	Many, immature	9.1

the standard dose found to give good growth of *Nematospora* by Buston and Pramanik (l.c.)). Equivalent quantities of inositol, of the inositol-free fraction, and of both fractions were also added to Medium A. These media were tested by the usual whole plate method and by the replaced segment method. Table I gives the results where whole plates were used and Plate X, Figs. 1, 2, and 3 illustrate the results obtained by the replaced segment method.

By both methods the results were very striking. Perithecia were formed in large numbers on media containing the crude lentil extract or the inositol-free fraction, but were not formed on Medium A or on that medium with the addition of inositol. The addition of inositol did not significantly increase the number of perithecia on media containing the second accessory factor. Thus, although both fractions had been shown to be essential for good growth of *Nematospora*, only the second accessory factor (or inositol-free fraction) was necessary for the formation of perithecia by the *Melanospora*. It must, therefore, be concluded, either that inositol is not essential for the formation of perithecia, or that *M. destruens* can build up this substance from a synthetic medium.

The effects of the lentil extract on the sporulation of a wide variety of fungi are listed below. The media used were Medium A, Medium A to which 0.2 per cent. crude lentil extract was added (referred to as Medium A + L), and in some cases, Medium A to which an equivalent quantity of the inositol-free fraction of the crude lentil extract was added (referred to as Medium A + F). Where other media were used these will be referred to in the appropriate place.

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(i) **BASIDIOMYCETES:** grown in large tubes at laboratory temperature, in the light. Media used: A, A + L, potato extract agar (P.E.A.) and potato extract agar + 0.2 per cent. crude lentil extract (P.E.A. + L).

*Armillaria mellea*; known to be a fungus which does not fruit freely in culture. In presence of lentil extract, growth better and small bulbils formed, which resembled young fructifications but did not develop further.

*Collybia velutipes*; fruited freely on all media used, rather less freely on A.

*Fomes fraxineus*; similar to the last.

*Hydnum coralloides*; fruited on all media but more freely on A + L and P.E.A. + L.

*Schizophyllum commune*; similar to the last.

*Sphaerobolus stellatus*; fruited on A + L and P.E.A. + L, but not on A or P.E.A.

(ii) **ASCOMYCETES:** plate cultures usually employed.

*Ascobolus denudatus*; produced apothecia freely on A + L in light, but not on A or A + F.

*A. viridulis*

*A. Leveillei*

*Saccobolus depauperatus*

} similar to last.

*Pyronema confluens*; fruited on A, but more freely on A + L. A + F slightly better than A but inferior to A + L.

*P. domesticum*; similar to last.

*Lambertella corni-maridis*; typical grey black pseudosclerotial crust (8) formed on surface of medium A + L seven days after inoculation, but not on A until seven days later. Six months after inoculation 82 fruit bodies on A + L (11 being mature, i.e. producing spores), only 1 immature fruit body on A.

*Sordaria fimicola*; sporulation poor on A, good on A + L and A + F. Result confirmed by replaced segment method (Pl. X, Fig. 4).

*Sordaria* sp.

*Rosellinia necatrix*

*Philocopra* sp.

} similar to last (Pl. X, Figs. 5 and 6).

*Melanospora zamiae*; growth and perithecial formation good on A + L, poor on A and A + F.

*Neurospora tetrasperma* (homothallic strain); perithecial frequencies twelve days after inoculation, A + L, 5.7; A + F, 3.9; A, few immature perithecia.

*Chaetomium cochliodes*; fruited equally freely on A, A + L, or A + F.

*Gymnoascus setosus*; similar to last.

With all the Ascomycetes listed above (with the exception of the last two) the lentil extract increased the number of ascus fructifications formed. The imperfect stage was favoured, however, with some other species.

*Aspergillus repens*; with media containing high concentrations of glucose, lentil extract caused great increase in conidial production, slight increase in perithecial production (i.e. greatly increased ratio of conidia to perithecia). With low concentrations of glucose, conidial production took place earlier in presence of 0.2 per cent. lentil extract.

*A. chevalieri*; similar to last.

*Ceratostomella adiposum*; pycnidia produced freely on A + L, sparsely on A or A + F.

*Isaria* sp.; coremia produced freely on A + L, not on A.

(iii) **ZYGOMYCETES:** plate cultures used.

*Mucor hiemalis*; plates inoculated with both + and - strains. Zygospores formed earlier and more freely on A + L than on A or A + F. A + F slightly better than A, greatly inferior to A + L.

*Phycomyces nitens*; plate inoculated with both + and - strains. No zygospores on A, few on A + F, numerous on A + L.

*Absidia glauca*; plates inoculated with both + and - strains, few zygospores on A, many on A + L and A + F.

*A. spinosa*; few zygospores on A, many on A + L and A + F, A + F being slightly inferior to A + L.

*Zygorhynchus Moelleri*; zygospores most numerous and formed earliest on A + L. Few on A, rather more on A + F. The following comparative values for perithecial frequency four days after inoculation were obtained: A, 1.9; A + F, 4.3; A + L, 19.3.

*Basidiobolus ranarum*; numerous zygospores on A + L, none on A or A + F.

(iv) OOMYCETES.

*Phytophthora erythroseptica*; no oospores on A or A+F, few on A+L. On transference to sterile water more oospores were produced on mycelium which had been growing on A+L than on that from A or A+F, possibly owing to the more vigorous growth of the former.

*P. cactorum*; no oospores on A or A+F, many on A+L. Results of transference to sterile water similar to those with *P. erythroseptica*.

*Pythium de Baryanum*; oospores formed more freely on A+L than on A or P.E.A.

*Trachysphaera fructigena*; oospores produced freely in liquid A+L, sparsely in liquid A or A+F. Conidia produced in large numbers on agar A+L, sparsely on A or A+F. Few oospores on agar A+L, none on A or A+F.

These experiments suggest that lentil extract contains some substance which has a stimulatory effect on the sporulation of a number of unrelated fungi. Some of the species tested (e.g. *Basidiobolus ranarum*, *Melanospora zamiae*) grew very poorly on Medium A and the increased sporulation on A+L might be correlated with increased growth. With other species, however, growth was good on A and was but little increased by the addition of lentil extract to the medium, whilst the increase in sporulation was very striking. A consideration of the mode of action of the lentil extract will be taken up in the next section with reference to *M. destruens*. It is hoped to carry out more detailed work on some of the species listed above later.

#### 4. MODE OF ACTION OF THE LENTIL EXTRACT.

A large quantity of crude lentil extract was made up by the method used by Buston and Pramanik (l.c.) described above.

While the effect on the sporulation of *M. destruens* obtained by adding 0.2 per cent. lentil extract to Medium A was very striking, it was realized that this effect might be due to the addition of some favourable food substance rather than to the presence of a specific accessory growth factor. The crude extract contains approximately 50 per cent. sugars and has a nitrogen content of 4-5 per cent. By adding 0.2 per cent. lentil extract to Medium A, therefore, one increases the amount of sugar in that medium and also the nitrogen content by approximately one-fifth. Such an addition of food substances might conceivably cause an increase in sporulation, but it is unlikely to cause such a large increase (see Table I). Moreover, it has been shown (1) that any increase in the glucose or potassium nitrate concentration of Medium A tends to reduce rather than increase perithecial formation. Phosphate and magnesium sulphate are known to be present in excess, since sporulation is equally good at lower concentrations of these substances and does not increase with increased concentration. It might be thought, however, that the increase in sporulation is due, not to increased concentration of food substances, but to the addition of some more suitable source of carbon or nitrogen. It was shown that this was not the case by growing the *Melanospora* on Medium A lacking one of its constituents and



on similar media to which 0.2 per cent. lentil extract was added. The results which are given in Table II show that the lentil extract is unable to replace either glucose or potassium nitrate. Thus the effects of lentil extract cannot be attributed to the addition of sugars or of a more favourable source of nitrogen, nor is it likely that the effects are due to the addition of phos-

TABLE II.

Medium used.	Perithecial frequency	
	8 days after inoculation.	12 days after inoculation.
A	0	0
A + L*	10.48	15.6
A - glucose	0.05	0.66
A - glucose + L	0.96	1.5
A - KNO <sub>3</sub>	0	0
A - KNO <sub>3</sub> + L	1.2	4.2
A - MgSO <sub>4</sub>	0	0
A - MgSO <sub>4</sub> + L	9.54	12.72
A - KH <sub>2</sub> PO <sub>4</sub>	0	0
A - KH <sub>2</sub> PO <sub>4</sub> + L	10.56	15.5
Plain agar	0	0.5
Plain agar + L	0.2	0.9

\* L = 0.2 per cent. lentil extract.

TABLE III.

Percentage lentil extract in medium.	Perithecial frequency	
	8 days after inoculation.	13 days after inoculation.
0	0	0
0.01	0	2.58
0.025	Some immature	4.24
0.05	Many immature	6.07
0.1	2.6	8.6
0.2	7.5	10.47
0.3	10.5	12.0
0.5	8.58	9.2
1.0	7.18	7.38

phate, since this has been shown to be present in excess in Medium A. The observed facts must therefore be interpreted as the result of the addition of a specific growth factor contained in the lentil extract.

Further evidence in support of this view was obtained by the use of media containing asparagin or ammonium nitrate as the source of nitrogen, to which 0.2 per cent. lentil extract was added. It had been previously shown (1) that asparagin was a less suitable source of nitrogen than potassium nitrate, while no perithecia were formed at all on media in which the sole source of nitrogen was ammonium nitrate. Perithecial frequencies of 9.2 and 8.8 respectively were obtained on media containing asparagin with 0.2 per cent. lentil extract and ammonium nitrate with 0.2 per cent. lentil extract. Since these figures are in excess of the value obtained when the sole source of nitrogen is the lentil extract (4.2, Table II) it must be concluded that the lentil extract has made possible the assimilation by the *Melanospora* of these normally unfavourable sources of nitrogen. Thus the extract acts as a true accessory growth factor.

The effects of concentration of lentil extract in the medium were examined and are illustrated in Table III.

The number of perithecia formed increased with increased concentration of lentil extract up to a concentration of 0.3 per cent., above which it slowly fell. The interpretation of this fall is not clear.

TABLE IV.

Percentage lentil extract.	Gm. glucose per litre of medium.	8 days after inoculation.	Perithecial frequency 13 days after inoculation.	20 days after inoculation.
0	40	0	0	0
"	20	0	0	0
"	10	0	0	0
"	5	0	0	0
"	2	0	0	2.1
"	0	0	0.86	1.22
0.025	40	0	Few immature	Few immature
"	20	0	" "	" "
"	10	Few immature	" "	" 2.48
"	5	Many immature	2.86	3.24
"	2	2.05	3.3	3.55
"	0	0.2	0.62	1.58
0.05	40	Few immature	Few immature	Few immature
"	20	" "	" "	" "
"	10	Many immature	3.2	3.75
"	5	1.7	4.5	5.0
"	2	3.3	5.18	5.6
"	0	0.58	0.6	1.3
0.1	40	Few immature	Few immature	Few immature
"	20	" "	" "	2.4
"	10	1.44	4.34	No increase
"	5	2.54	7.02	" "
"	2	1.94	4.04	" "
"	0	0.5	1.6	" "
0.2	40	5.58	No increase	" "
"	20	9.77	" "	" "
"	10	14.22	" "	" "
"	5	10.48	" "	" "
"	2	6.075	" "	" "
"	0	1.86	2.1	" "

TABLE V

Percentage lentil extract in medium	0	0.025	0.05	0.1	0.2
Optimum amount of glucose in grammes per litre	1.2	2	2	5	10
Maximum amount of glucose in grammes per litre	2.5	10	10	20	40

The relation between glucose content of the medium and the amount of lentil extract added was studied in some detail. The results of a typical experiment are given in Table IV.

This Table shows that by increasing the amount of lentil extract in the medium the maximum and optimum amounts of glucose for perithecial formation can be greatly increased. This fact is brought out more clearly by the following rearrangement of data from Table IV.

The lentil extract thus either enables the fungus to sporulate in the presence of a higher concentration of glucose or, by increasing the growth of the fungus, enables it to use the glucose more rapidly and in greater quantity, thus bringing the concentration down to a region suitable for sporulation.

A somewhat similar result was obtained by varying the nitrate concentration of the medium. The increase in the optimum nitrate concentration with increase in percentage lentil extract present was less pronounced, however, than in the case of glucose.

The fact that the active spore promoting substance was ether-insoluble was verified by experiment.

#### 5. COMPARISON OF EFFECTS OF LENTIL EXTRACT WITH THOSE OF CERTAIN FUNGAL PRODUCTS.

The spore promoting substance present in the crude lentil extract and that previously shown to be produced by certain fungi (1) thus possess a number of similar properties. Both substances are ether-insoluble, both show increased effect with increased concentration up to a point and the addition of sufficient glucose reduces the stimulatory effect in both cases.

A detailed comparison of the effects of the two substances on the sporulation of *M. destruens*, *Sordaria fimicola*, *Rosellinia necatrix*, and *Zygorhynchus Muelleri* in the presence of different amounts of glucose was undertaken.

*Botrytis cinerea*, *Fusarium fructigenum*, and an unidentified bacterium were grown for fourteen days at laboratory temperature in liquid Medium A. The staled liquid was then filtered and used as a source of the growth substance.

The effects on sporulation of *M. destruens* of two samples of lentil extract were compared with those of two lots of medium staled by *B. cinerea*, two by *F. fructigenum*, and one by the bacterium. In these experiments the object was to determine how far different additions of active substance affected the sugar concentration which is optimal for sporulation. With fungal staled media as sources of the active principle, the constituents of Medium A with varied amounts of glucose were added to the staled medium, which was undiluted, diluted, or concentrated as the case may be. In the scale of arbitrary units given in the top row of Table VI, the undiluted staled medium is given the value 1. With lentil extract as the source of the active substance, various amounts were added to Medium A, the glucose content of which was also adjusted to various levels. In this case the arbitrary unit represents 0.1 per cent. (dry weight) of lentil extract.

The figures in the table represent the glucose concentration (in grammes per litre) optimal for sporulation under the various conditions.

The amount of residual glucose present in the media staled by *Botrytis* or *Fusarium* was estimated and the figures in the table are adjusted accordingly. The glucose content of the medium staled by the bacterium and that of the lentil extract were disregarded. Hence in these two cases the

TABLE VI.  
(For explanation see text.)

Source of active substance.		Concentration of active substance in arbitrary units.						
	o.	1/30.	1/10.	1/4.	1/2.	1.	2.	3.
<i>Melanospora destruens.</i>								
Botrytis medium I	I	—	—	—	3	4	9	—
" " 2	"	—	I	—	2	3	6	II
Fusarium " I	"	—	2	—	2	2	5	—
" " 2	"	I	—	—	2	5	5	—
Bacterium " I	"	—	—	—	I	2	5	—
Lentil extract I	"	—	—	—	2	2	5	—
" " 2	"	—	—	2	2	5	10	—
<i>Sordaria fimicola.</i>								
Botrytis medium	2	—	2	—	3	4	6	—
Lentil extract	"	—	—	—	2	3	5	—
<i>Zygorhynchus Moelleri.</i>								
Botrytis medium	I	—	2	—	3	4	6	8
Fusarium "	"	—	I	—	I	2	5	—
Bacterium "	"	—	—	—	I	I	2	—
Lentil extract	"	—	—	—	5	5	10	—

figures for glucose concentration are probably slightly too low, particularly with higher concentrations of the stimulatory substance.

It is clear from a consideration of that part of Table VI which relates to *M. destruens* that the active principles from lentil extract and staled media showed a parallel behaviour in the presence of varying quantities of glucose, viz. with increased concentration of stimulatory substance the concentration of glucose optimal for perithecial formation was raised.

Similar results were obtained with *S. fimicola* and *Z. Moelleri* and are summarized in the table. *R. necatrix* gave comparable results, but these were less decisive, owing to the extreme sensitivity of this fungus to toxic staling substances.

The active substances have so far been compared from the point of view of their effects on the sporulation of certain fungi. It remains now to compare their effects on growth itself. For this purpose the fungus *Nematospora gossypii*, which does not grow in the absence of accessory growth factors, was used as the test object. The work of Buston and Pramanik (l.c.) had shown that lentil extract is an excellent source of these substances for this fungus. In the light of the foregoing account it was, therefore, of interest to see how far the growth requirements of *Nematospora* could be satisfied with fungal staled media or mycelial extracts.

The basal medium used by Buston and Pramanik for the culture of *Nematospora* had the composition: glucose, 0.25 per cent.;  $K_2HPO_4$ , 0.5 per cent.;  $MgSO_4$ , 0.25 per cent.; hydrolysed gelatin to give 0.3 per cent. N; agar 1.5 per cent. These ingredients were added to liquid

TABLE VII.

Medium used.		Growth of mycelium (mg. dry wt.).
*Basal		Negligible
* " + 0.05 % lentil extract		60
* " + 0.1 % " "		130
* " + 0.2 % " "		280
* " + inositol fraction corresponding to 0.2 % lentil extract		20
* " + inositol-free " " " "		18
* " + both fractions " " " "		285
* " + 0.4 % lentil extract		330
" in <i>Fusarium</i> staled liquid		Negligible
" " " " + inositol		10
" " <i>Botrytis</i> " " "		5
" " " " + inositol		27
" " extract of <i>Botrytis</i> mycelium		177
" " " " + inositol		238

\* Data from Buston and Pramanik (l.c.).

Medium A which had been staled by *B. cinerea* or *F. fructigenum*. In some cases an amount of inositol corresponding to 0.2 per cent. lentil extract was also added. In another series of experiments the constituents of Buston and Pramanik's medium were added to a watery extract of fungal mycelium prepared as described above (p. 700).

The results of representative experiments from this series are given in Table VII. Data, taken from Buston and Pramanik's paper, relating to the effects of various concentrations and fractions of lentil extract are given in the table for comparison.

Growth of *N. gossypii* on media prepared from liquids staled by *Fusarium* or *Botrytis* was poor and the addition of inositol led to very little improvement. This poor growth may have been due to low concentration of the accessory growth factors or to the presence of staling products. The latter is more probable, since the addition of lentil extract to the medium did not significantly increase the growth of the fungus.

Mycelial extracts, however, to which the ingredients of the basal medium were added gave fairly good growth of the *Nematospora*. The addition of inositol to such a medium gave results comparable to those obtained with lentil extract. It was concluded that, while the mycelial extract contained an amount of the second accessory factor comparable to that in a medium containing 0.2 per cent. lentil extract, it was rather less rich in inositol than was the latter.

Thus the accessory growth factors present in lentils and those produced by certain organisms show a very close similarity in their effects on the

sporulation of certain fungi and on the growth of *N. gossypii*. It is, therefore, probable that these active substances are the same, or at least very similar.

## 6. DISCUSSION.

The experiments described in this and the earlier paper have a direct bearing on mycological technique in that they indicate a method of increasing and accelerating spore production. With *M. destruens* the effect of adding a small quantity of lentil extract to a synthetic medium is to cause sporulation to begin on the fourth instead of the twenty-first day after inoculation and to increase the number of perithecia formed by approximately three times. The effect is relatively greater on certain other artificial media which give no sporulation of *Melanospora* at all, but which are quite suitable for this purpose if some of the extract be added. A similar behaviour is shown by a number of other fungi (see p. 702), perhaps the most striking case being that of the Discomycete *Lambertella cornimaris* which produced only a single fruit body on a synthetic medium but as many as eighty-two when lentil extract was added. Some fungi, such as *Hydnum coralloides* and *Pyronema confluens*, spored freely on the synthetic medium, but nevertheless the addition of lentil extract gave a measurable improvement. Others, such as *Chaetomium cochliodes*, sporulated strongly on the synthetic medium and showed no response to the addition of lentil extract. At the opposite extreme is such a fungus as *Armillaria mellea*, which produced fruit bodies neither on the synthetic medium nor on the same with the addition of lentil extract. Nevertheless the addition of the latter appeared to carry the fungus a stage nearer to fructification (see p. 702).

It can reasonably be claimed that the addition of lentil extract or of certain fungal products to the culture medium increases the tendency to sporulation. The actual effect obtained would be expected to be most striking when the conditions are such that sporulation takes place tardily or feebly in the absence of the extract. In many cases the addition of the extract then induces sporulation to take place quickly and freely. Where sporulation readily occurs in the absence of the extract, there is obviously little room for improvement, and where, as with *Armillaria mellea*, sporulation is not achieved even in the presence of the extract, one may suppose that some other factor is not properly adjusted and that the case requires further particular investigation.

Before proceeding to discuss the mode of action of these extracts upon growth and sporulation, it may be desirable to emphasize again that they do not function simply by supplying suitable ingredients to a synthetic medium which is made up of substances unsuitable for growth. This point has already been fully discussed (p. 704) with respect to the effects on *M. destruens*. Farries and Bell (l.c.) have shown that the addition of certain

extracts to a basal synthetic medium stimulates the growth of *Nematospora* to an extent far beyond that on either of the components, and more particularly that the addition of the extracts enables that fungus to assimilate certain substances (e.g. asparagin, gelatin) which otherwise it is unable to use. One is justified, therefore, in speaking of these extracts as containing accessory growth substances.

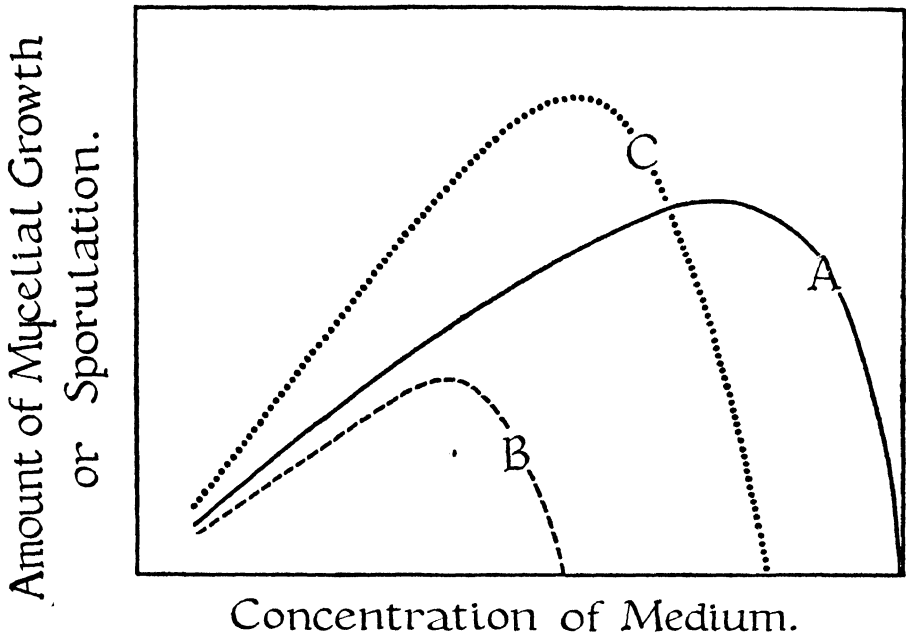
The interpretation of the experimental results in this study meets with considerable difficulties. In the first place the active principle contains at least two constituents and it is not certain that these are equally important in all cases. Certain fungi are able to produce these substances in the course of their growth, some being more active than others in this respect. Hence when the addition of the accessory factors from an outside source (e.g. lentil extract) makes no appreciable difference to the behaviour of the fungus it is not immediately clear whether the latter does not require these substances or whether it is able to produce the necessary amount by its own processes. Finally, the active principles act both upon growth and upon sporulation, but not in the same manner upon each. It will aid to clearness to deal separately with the effects upon growth and sporulation.

*Growth.* *N. gossypii* may be taken as a good example of the kind of fungus which is entirely dependent upon an outside source of accessory substances for growth. Both accessory factors (viz. *i*-inositol and the second factor) are equally necessary. At the opposite extreme are such fungi as *B. cinerea*, *F. fructigenum*, and many others which grow perfectly satisfactorily on a simple synthetic medium. It is known that *B. cinerea* growing on a synthetic medium produces both accessory factors, since extracts of *Botrytis* mycelium when added to a synthetic medium allow of vigorous growth of *Nematospora*. The growth-promoting substances are also excreted by *Botrytis* into the cultural medium, but the latter is not a suitable source of them for the culture of *Nematospora* on account of the simultaneous presence of staling substances. *M. destruens* represents a type intermediate between the foregoing extremes. On a shallow layer of synthetic medium (Medium A without agar) it makes no growth, but it grows well if 0.05–0.2 per cent. lentil extract or an equivalent amount of the inositol-free fraction of the extract be added. The addition of inositol is not necessary. At the moment it is not clear whether the fungus does not require inositol or whether it produces it in sufficient quantity of its own accord. A scanty growth of the fungus takes place when the synthetic medium is solidified with agar, a result which is most readily interpreted as indicating the presence of traces of the second growth factor in the agar.

There are many fungi which behave somewhat similarly to *Melanospora*. On a synthetic medium with agar they produce a poor type of growth, which is much increased by the addition of the accessory substances.

Presumably one or other of the latter is lacking or is produced too slowly by the fungus to allow of vigorous growth.

*Sporulation.* The effect of the growth-promoting substances upon sporulation will be considered in relation to the Text-figure, which represents



Diagrammatic representation of the influence of concentration of the medium on growth and sporulation. Curve A represents growth, Curve B represents sporulation, and Curve C represents sporulation in the presence of an additional source of the accessory growth factors.

diagrammatically the influence of concentration of nutrient medium on growth and sporulation. Increase in concentration of the medium up to a point increases both growth and sporulation, but beyond that sporulation falls off whereas mycelial growth continues to increase up to a concentration at which, presumably, osmotic factors begin to exert a depressing effect. The exact positions of the optima vary, of course, very considerably for different fungi.

This general relationship was emphasized by Coons (6) who advocated the use of a rather dilute medium if good sporulation were the object in view. A similar conclusion was reached by Brown (2) in his study of the factors which control the sporulation of *F. fructigenum*. Spore formation in that fungus is preceded by autolysis of the vegetative mycelium, and at concentrations of the medium above a certain point autolysis is progressively checked, the vegetative mycelium is persistent, and sporulation is depressed and finally inhibited.

The relation between growth and sporulation is therefore complex.



Up to a certain point there is a positive correlation between the two, but at higher concentrations this gives place to a negative correlation. Interpretation of the effects of growth factors on sporulation will thus be different according as one is considering the lower or higher regions of concentration.

At lower concentrations of the media, where both curves are ascending, the stimulating effect of the accessory factors upon sporulation may be referred, in part at least, to their effect upon growth. More mycelium is produced and, the medium not being too concentrated for sporulation to proceed, more spores are accordingly formed. When, however, the two curves diverge, another explanation has to be found, and an indication of this has been discovered, viz. that there is an antagonism in respect of sporulation between nutrient substances (more particularly the carbohydrate constituent) and the accessory growth factors.

Addition of the growth factors to a synthetic medium increases the sporulation of *Melanospora* (and of other fungi) but this beneficial effect does not arise if at the same time a sufficient amount of glucose be added. The data of Tables II and III illustrate clearly the effects of various additions of active principle on the amount of sporulation. In the absence of lentil extract the optimum concentration of glucose in the standard medium was 0.2 per cent. By the addition of the standard dose of extract the optimum concentration is raised to 1 per cent. The effect is therefore to prolong the up-grade part of the sporulation curve as is illustrated in the Text-figure (Curve C as against Curve B).

The results of this study, so far as they concern sporulation, can be interpreted on the following hypothesis. Three factors within the cultural medium influence sporulation: the concentrations of food substances, of accessory growth factors, and of staling substances. It is suggested that the stimulation to sporulation arises from a low ratio of food substance to accessory factors, provided that the concentration of staling substances has not become high enough to interfere seriously with metabolism. The relations between certain types of media and sporulation will be briefly considered in the light of this hypothesis.

The relatively low concentration of food substances which is optimal for sporulation would be explained as follows. In a somewhat concentrated medium, conditions in the early stages are quite unfavourable for sporulation. The concentration of nutrients is high and, unless a special addition of accessory factors has been made, the concentration of the latter is low. As a result of growth the former diminishes and the latter increases. There is, however, a simultaneous increase of staling substances. The nature of these depends very largely upon the original composition of the medium and upon the particular organism, but it is reasonable to suggest that in a somewhat rich medium staling factors have come into play and are severely

depressing all metabolic activity by the time that the ratio of nutrients to accessory growth substances has become suitable for sporulation. From a consideration of the interaction of these three factors, the fact that the concentration of nutrients optimal for sporulation is below that optimal for mycelial growth can be easily understood.

Natural media, such as malt agar, contain appreciable amounts of the accessory substances, and in accordance with this fact tend to be favourable for both growth and sporulation. Any increase of the concentration of such a medium increases both the ordinary food constituents and the accessory substances, but ultimately the effect is the same as before, viz. a repression of sporulation. In the case of malt agar the concentration optimal for the formation of perithecia by *M. destruens* is 3 per cent., but this optimum can be raised or lowered by the addition of lentil extract or glucose respectively.

The favourable effects on sporulation which are observed when the culture medium is diluted in an appropriate manner can also be readily interpreted in the light of the hypothesis. If on a certain medium growth has proceeded so far but sporulation has not begun, this may be ascribed to the concentration of food substances still remaining too high or to staling factors having become too pronounced. If now these are diminished, as by allowing the fungus to grow on to a weaker medium or by bodily transference to water, sporulation is stimulated to begin.

One may therefore suggest that the principle governing sporulation is that the fungus by its growth should decrease the concentration of nutrients below a certain point and increase that of the accessory substances above a certain point before growth-inhibiting or staling factors have become too pronounced. This favourable state of affairs is more likely to be reached if the medium be made up with a surplus of accessory substances at the start. In the light of what is known of the antagonistic relationship, as regards sporulation, between food substances and accessory factors, it would be expected that the addition of the latter would induce the fungus to sporulate in the presence of a higher concentration of food substances than would otherwise be possible; in other words, one would expect a marked acceleration of sporulation. This acceleration, rather than the actual increase in amount of sporulation, is perhaps the more notable of the effects produced by the addition of accessory growth substances to the culture medium.

At the moment one can refer only in the broadest terms to the concentration of the accessory substances present in a given culture. A certain amount may be added to the initial medium, but it is not known how quickly this is absorbed by the growing mycelium. Moreover, various fungi produce one or both of the accessory substances in different degree. As long as a surplus of food substances is present, one may suppose that the accessory substances are utilized for growth, but whether they are lost, temporarily or permanently, in the process is not known. One has simply the

experimental fact that by supplying a quantity of accessory substances to the medium, one produces an acceleration and intensification of fruiting, so that presumably there is a higher concentration of those substances in and around the fungal hyphae than would otherwise have been the case. To this higher concentration one may ascribe the earlier and more intense sporulation.

It is probable that the accessory factors are present in varying degree in a wide variety of plant or animal material. Buston and Pramanik (l.c.) obtained them not only from lentils but from Lupin seedlings, yeast, ox heart, and, in smaller quantity, from cotton leaves and potatoes: Farries and Bell (l.c.) had previously demonstrated their presence in egg white, milk casein, and commercial peptone. In the present paper they have also been shown to be present in malt extract, in fungal mycelium, and in media in which certain fungi had been grown. The writer has also found spore-promoting activity in preparations of oatmeal and in a carrot extract (prepared in the same way as the lentil extract and found by Padwick (15) to stimulate the growth of *Ophiobolus*).

Farries and Bell (l.c.) compared the accessory factor necessary for growth of *Nematospora* with the 'Bios' substance of Wildiers (l.c.), with a bacterial growth-promoting substance described by Reader (16) and with Vitamin B. Buston and Pramanik (4) carried the comparison with 'Bios' rather farther and concluded that the two substances were not the same. They were also able to demonstrate (3) a dissimilarity between the effects of their preparations and those of Reader (l.c.) which suggested that the active principles concerned were different.

It was shown by Lepeschkin (10) as early as 1924 that growth of *Penicillium glaucum* could be increased by the use of Vitamin B preparations. More recently Schopfer (17, 18) reported that the addition of Vitamin B or of extracts of germinating wheat grains to the medium promoted growth and zygosporangium formation in *Phycomyces blakesleanus*. Experiments by the present writer with a small sample of a Vitamin B<sub>1</sub> preparation showed that it was not so effective as lentil extract in promoting sporulation of *Melanosporea* and that it was distinctly poor in its effect upon the growth of *Nematospora*. At the moment it does not appear as if the two active principles are the same.

A comparison can more profitably be made with Nielsen's 'growth substance B'. Nielsen found that the medium in which *Rhizopus suinus* had been grown promoted the growth of yeast (11) and of *Aspergillus niger* (12). Later, Nielsen and Hartelius (14) showed that such a medium contained both an ether-soluble substance, capable of promoting the growth of oat coleoptiles, which they termed 'growth substance A' (later termed hetero-auxin and identified as  $\beta$ -indolyl-acetic acid (9, 19)) and an ether-insoluble substance, which when added to a synthetic medium increased the growth of a yeast and of *Aspergillus niger*, and which they termed

'growth substance B'. The formation of conidia by *Aspergillus* was also stimulated by the latter substance. Nielsen (13) also demonstrated growth-promoting activity in beerwort. Growth substance B resembles the accessory factors from lentils and those from the fungal products used in the present investigation in being ether-insoluble, but is said to be precipitated when added to ten volumes of 96 per cent. alcohol. Similar fractionations of a mycelial extract of *Botrytis* and of medium staled by that fungus were carried out by the writer. Both the substances promoting sporulation in *Melanospora* and those necessary for the growth of *Nematospora* remained in the filtrate. It is probable that the method of fractionation used was slightly different from that of Nielsen and Hartelius, or the divergence may be due to the use of culture media of different composition. Nielsen and Hartelius also state that growth substance B is insoluble in absolute alcohol and but slightly soluble in 95 per cent. alcohol. A similar result was obtained by Farries and Bell (l.c.) for the accessory growth factors obtained from egg white. These authors also found that when the active extract was added to three volumes of 95 per cent. alcohol the accessory substances remained in the filtrate.

Thus the various substances promoting growth and sporulation of fungi which are described here and elsewhere, while possessing a general similarity in behaviour, have been shown to differ in certain respects. One may suggest that it is possible to emphasize unduly the small differences shown, which may arise from variation in technique or from various amounts or kinds of impurity present. The several growth substances may be the same, but, as in the case of some enzymes, their properties in detail may be modified by the presence of other substances.

## 7. SUMMARY.

1. A crude extract of lentils, which had been shown by Buston and Pramanik (3) to stimulate the growth of *N. gossypii*, was also found to stimulate sporulation of *M. destruens* and of a number of other fungi.

2. The active substance was previously fractionated by Buston and Pramanik into *i*-inositol and an inositol-free fraction (second accessory factor), both of which were essential for growth of *Nematospora*. It was found that the addition of inositol was unnecessary for sporulation of *M. destruens* and of some other fungi. A few of the fungi examined required the addition of both factors before fruiting took place.

3. The stimulatory effects of the lentil extract were not due to the presence of suitable carbohydrates or nitrogenous food substances, since neither the glucose nor the potassium nitrate of the basal medium could satisfactorily be replaced by the extract.

4. Sporulation increased with increasing amounts of extract added, up to an optimum.

5. In the presence of lentil extract the concentrations of glucose in the basal medium which were optimal and maximal for the formation of perithecia by *M. destruens* were appreciably raised.

6. A detailed comparison was carried out of the effects of the active substances from lentils and from fungal products on the sporulation of *M. destruens*, *Sordaria fimicola*, *Rosellinia necatrix*, and *Zygorhynchus Moelleri* and on the growth of *N. gossypii*. The active substances from both sources behaved similarly, but the fungal preparations were less rich in inositol than was the lentil extract.

7. Conditions governing sporulation in fungi are discussed, and a comparison is made between the active substances investigated and certain other growth-regulators described elsewhere.

The writer is indebted to Professor W. Brown for suggesting this work and for assistance in the formulation of the theoretical views expressed in this paper.

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## EXPLANATION OF PLATE X.

Illustrating Dr. Hawker's paper on 'The Effect of Certain Accessory Growth Substances on the Sporulation of *Melanospora destruens* and of some other Fungi'.

The perithecia are shown as small black objects which can be clearly seen with the aid of a hand lens. The Petri dishes used were 10.5 cm. in diameter.

Fig. 1. *Melanospora destruens*, seven days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + 0.2 % lentil extract.

Fig. 2. *M. destruens*, seven days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + equivalent amount of inositol fraction of lentil extract.

Fig. 3. *M. destruens*, seven days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + equivalent amount of inositol-free fraction of lentil extract.

Fig. 4. *Sordaria fimicola*, ten days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + 0.2 % lentil extract.

Fig. 5. *Sordaria* sp., ten days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + 0.2 % lentil extract.

Fig. 6. *Rosellinia necatrix*, ten days after inoculation. Original medium in plate = Medium A; medium in segment = Medium A + 0.2 % lentil extract.

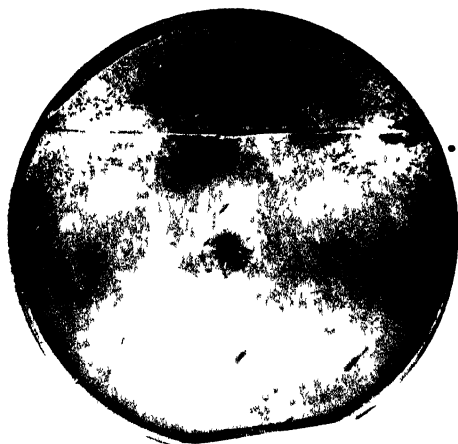




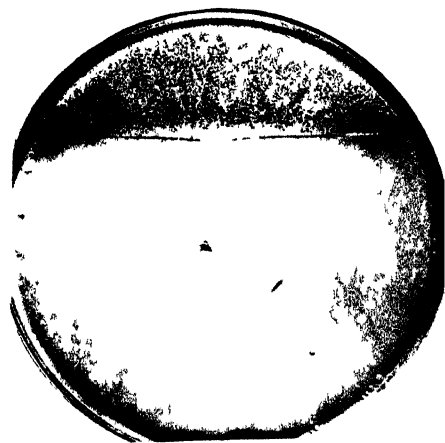
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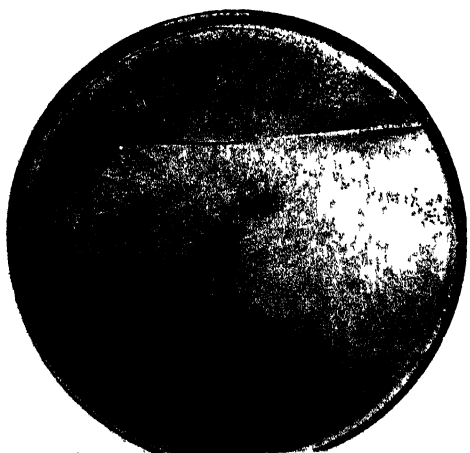
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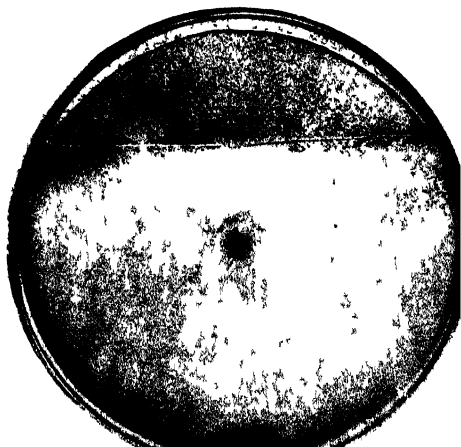
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# Observations on Inheritance in *Coprinus macrorhizus* (Pers.) Rea.

BY

HUGH DICKSON.

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With Plates XI and XII and two Figures in the Text.

## I. INTRODUCTION.

SOME years ago, during an investigation of the effect of X-rays on fungi, a peculiar type of saltant sector was obtained on irradiating a haploid colony of *Coprinus macrorhizus*. Owing to the stock cultures becoming abnormal after several months in pure culture it was not possible to continue the investigation at that time. Recently, however, a fresh stock of the fungus was obtained, the experiment repeated (the results corroborated those previously obtained) and the investigation pursued.

The segregants from a cross between an X-ray induced saltant and a normal haploid have been examined and the results compared with those previously obtained in experiments on *C. sphaerosporus*.

Following the results on the interactions of diploids and haploids obtained with the bipolar *C. sphaerosporus* (2 and 3), similar experiments have been carried out using artificially induced mutant types for comparison with the morphologically homogeneous haploids of the tetrapolar *C. macrorhizus*. These experiments have brought to light certain facts concerning the reactions associated with 'illegitimate' matings originally described by Buller (1) for *C. lagopus*.

## II. EXPERIMENTAL PROCEDURE.

The procedure adopted with the two strains of *C. macrorhizus* was similar, and consisted primarily in obtaining pure cultures of the diploid mycelia which were then used to inoculate sterilized horse-dung in jars. The jars were incubated at 25° C. until fruit-body rudiments became apparent, when they were placed on a bench near a window as light was found to be necessary for the development of the fructification as in the case of *C. lagopus*. Single spore colonies were obtained by pouring an aqueous suspension of ripe spores over horse-dung agar medium contained

in Petri dishes, draining off the superfluous liquid and incubating the dishes at 25° C. for twenty-four hours, by which time most spores had germinated. Single colonies were located under a binocular microscope and were removed to dung-agar slopes with the aid of a fine needle. The haploid colonies so obtained were then grown together in various combinations in pairs and were found to be separable into four 'sex' groups. These will be referred to as strains *AB*, *ab*, *Ab*, and *aB*, of which the first two and the last two strains combine respectively to give diploid mycelia.

In the case of *C. sphaerosporus* (2, p. 184), it was found that spore germination on malt-agar approached 100 per cent., but that a varying percentage of the sporelings were dwarfs, growth ceasing when the colony was only a fraction of a millimetre in diameter. The majority of spores of *C. lagopus* germinated on malt but a higher percentage germination was obtained on dung. *C. macrorhizus*, on the other hand, was found to germinate very poorly on malt, the percentage germination being probably under 40 per cent., whereas on dung practically all the spores germinated in every strain examined. This difference in the responses of *C. macrorhizus* and *C. lagopus*, is of interest in view of the close similarity existing between the two species, *C. macrorhizus* being originally described as a variety of *C. lagopus*.

A malt-agar medium (2 per cent. malt, 2 per cent. agar) was found to be more suitable than dung for purposes of comparison of different morphological types and was used accordingly, otherwise dung-agar was used throughout, as on this medium the various strains retained their original characteristics (rate of spread, appearance, and readiness to diploidize one another) better than on malt. It was found, however, that following several months in pure culture some haploids tended to change even when kept on dung-agar. The alteration in all cases was in the same direction, namely, a slight decrease in growth-rate accompanied by a denser type of aerial growth in which the hyphae grew close to the surface of the medium. Such strains, while retaining their sexual reactions unaltered, were slower in forming diploids when mated together than were those having the original phenotype. Whether this change was due to mutation or to a slow and gradual alteration was not determined. Sectors of the type were not observed and sectoring of any kind was not of frequent occurrence.

Malt-agar was used as the culture medium for colonies which it was proposed to irradiate. The colonies were allowed to grow until they reached the periphery of the dish, when they were exposed to a dose of X-rays which varied from 70 to 95 K.V. (rectified) at a tube current of 5 m.a. and a distance from the target of some 20–35 cm. A celluloid filter was used in all cases, and the length of exposure was from three to four hours. Cultures irradiated for three hours at 70 K.V., 5 m.a., 35 cm. distance received 11,413, r-units. The 'harder' radiation produced at the

higher potential gave rise to no qualitative differences in the results, but the number of saltants produced was greater with the increased dose. Following irradiation each colony was cut into about twenty squares, which were used to inoculate a similar number of Petri dishes containing malt-agar, these were then incubated at 25° C. and allowed to grow for several days before they were examined.

### III. RESULTS OF TREATMENT WITH X-RAYS.

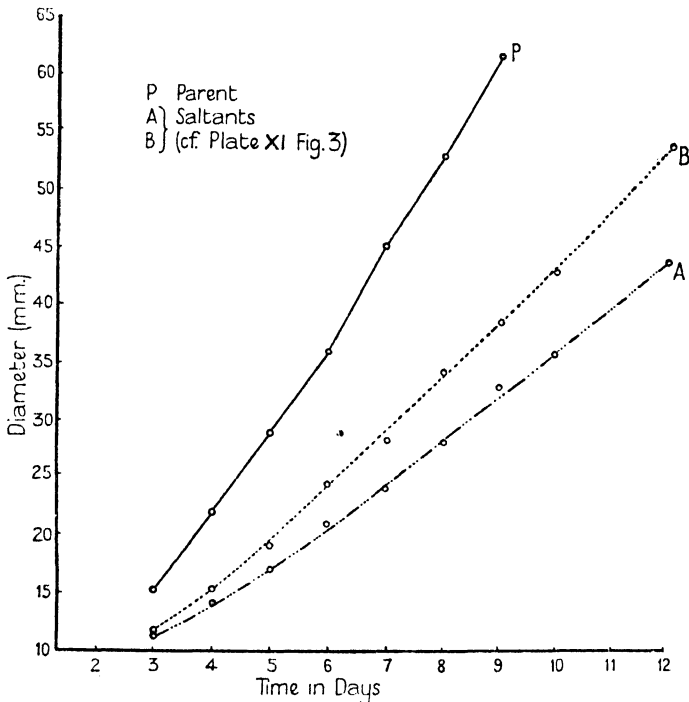
Several haploids of each sex were irradiated in both the first and second strain of *C. macrorhizus*. In each strain the reactions were similar in three of the four sexes. Whether colonies of the fourth sex of each strain were of the same sexual group, is unknown. It was established, however, that the saltant *A*, of the fourth or unique sex of the first strain paired (after some three years in culture) with cultures of all four sexes of the second strain. The distinction between the three similar sexual strains and the fourth strain lay in the different types of saltant sectors produced as a result of irradiation. In strains of all sexes there were saltants of various common types, generally, though not always, producing a more plentiful aerial mycelium than the parent strain. In some cases they grew faster than the parent when the 'arms' of the sector diverged at an increasingly wide angle from one another (Pl. XI, Fig. 1). When the rates of parent and saltant were equal the arms were approximately straight (Pl. XI, Fig. 2). When the saltant grew more slowly than the parent (Pl. XI, Fig. 3, *B*) the latter generally surrounded the former. These results were obtained from a measurement of the growth-rates of ten different saltants which had been subcultured from sectors and grown from single hyphal tips, and a comparison with the rates of spread of the parent types (all of which were approximately identical).

The fourth sex (called *aB* in both strains), in addition to the types of saltant found in the other sex strains, produced a sector which displayed certain peculiarities. On its first appearance it formed a sector with increasingly diverging arms and its periphery extended beyond that of the parent culture. In all cases it had considerably more aerial mycelium than the parental type. Pl. XI, Figs. 4 and 5, show such sectors at an early stage, Figs. 6, 7, and 8 at an intermediate stage, and Fig. 3, *A*, at a late stage of development. All the sectors show the same peculiarity but not always to the same degree. In each case the origin of the sector can be traced to approximately the centre of the colony and it will be seen that the distance from the centre to the periphery at a point immediately opposite the origin is less than the distance to any other point on the periphery of the saltant. This might be otherwise expressed by saying that there is a 'dip' in the periphery of the sector at the point opposite its origin. This 'dip' can be seen even in young cultures such as those of Figs. 4 and 5, though it becomes more

pronounced at later stages. Saltants such as these had in general two forms, namely, that shown in Fig. 6 and that in Figs. 3, 8, and 9, whether these are modifications of a common type is not known with certainty, but from a comparison of a number of such saltants it appears probable. Fig. 3 shows the latest stage which has been grown of one of these saltants and it can be seen that the arms of the sector after diverging at an increasing angle finally begin to converge. It is not known, however, whether this generally happens at such a stage. In most of the older cultures a well-marked ridge develops where saltant and parent meet (cf. Figs. 3, 7, 8, and 9), and in some cases at the periphery of the sector a very thin mycelial growth can be seen (Figs. 3, 8, and 9). Subcultures from this have been taken, the cultures purified and the growth-rates determined. Text-fig. 1 shows the growth-rate of the parent strain and of saltants *A* and *B* of Pl. XI, Fig. 3. It is seen that while saltant *A* has the slowest growth-rate it formed a diverging sector, whereas saltant *B*, whose growth-rate was faster than *A* but slower than the parental colony, was gradually surrounded by the latter. Results similar to those found with strain *A* have been obtained with other saltants of similar type.

Strain *A* and another similar saltant of strain 16, namely 16.1, were each grown together with their respective parents in the same dish. In each case both the saltant and its parent grew normally up to the time when the two mycelia met (cf. strain *A* and its parent, Pl. XI, Fig. 10). Shortly after the two colonies had come into contact the saltant developed a fluffy white mycelium at the margins of the colony adjacent to the parent (Fig. 11). The areas of plentiful aerial mycelium spread gradually round the periphery of the saltant till they met and at the same time they developed centripetally though never completely reaching the centre of the colony. (Figs. 12 and 13 strain *A* and parent, and Fig. 16, strains 16.1 and 16). At a later stage (Figs. 14 and 17) it was found that the saltants reverted to their normal thin mode of growth. This reversion occurred first at the section of the colony furthest from the parent. In Fig. 14 the dark sector extending from the centre of the colony to the periphery is probably a saltant. An inoculum taken from the fluffy area of either saltant gave rise to a thin mycelium indistinguishable from the original saltant. It can be seen in Figs. 13, 14, and 17 that the line where the saltant and parent meet is approximately straight and in each case that the two strains have about equal diameters, both facts indicating that the growth-rate of the saltant has been increased by contact with its parent. When the saltants (both of sex *aB*) were paired with other strains of the same sex somewhat similar results were obtained to those already described. When they were paired with strains of opposite sex to their parents (namely *Ab*) both strains were diploidized (Fig. 20). When paired with either of the remaining sexes *AB* (Figs. 18 and 19) or *ab*, the results were

on the whole similar to those with strains of sex *aB* except that after a time the line where the strains met was no longer straight but curved, the curve being convex towards the parent culture. While the above description indicates the general course of the reaction there was considerable variation in detail especially as regards the shape of the line where the cultures met.



TEXT-FIG. 1. For explanation see text.

In all cases the saltant became fluffy. Neither the saltant nor the parental strain with which it was mated produced clamp connexions except where the two strains were of opposite sex.

As it was obvious from the preceding experiments that there was some reaction between the saltants and the normal strains the following experiments were performed to elucidate if possible its nature. Strains 16,1 and 16 were placed as before about a centimetre apart in a Petri dish, but were separated by means of a thin quartz or glass coverslip placed vertically and penetrating the medium to the bottom of the dish. Although the cultures were kept for several days after the strains had severally reached the coverslip no effect on the saltant was observed. It was concluded that the stimulus was not of the nature of a radiation capable of penetrating quartz or glass. Two Petri dishes, one containing a culture of 16,1 and the other of 16 were placed face to face with the lids removed and adjusted so that the colonies were as near to one another as possible while at the same

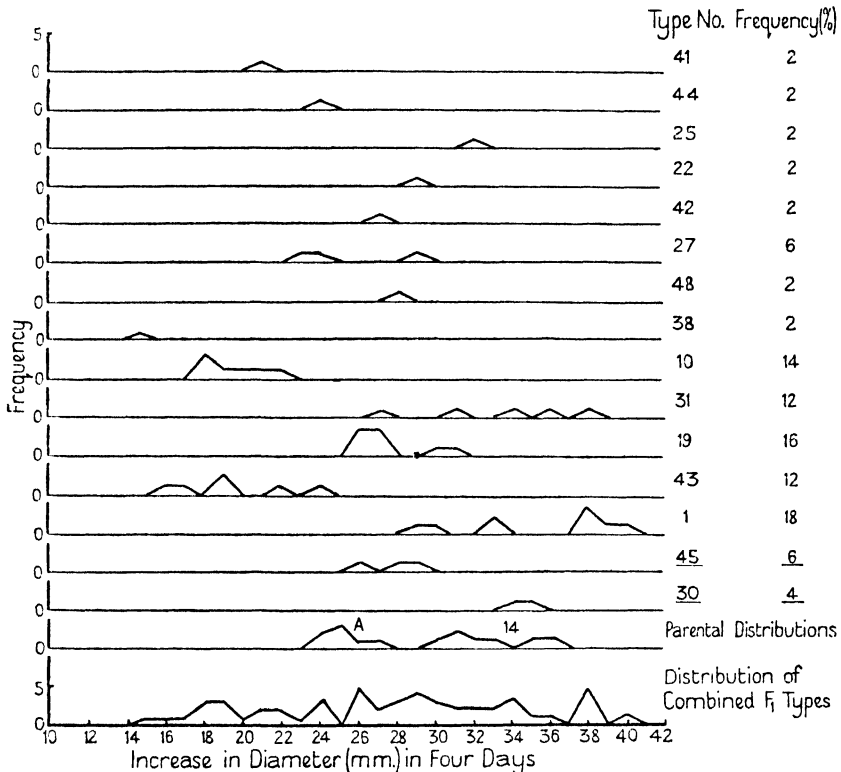
time the mycelia did not touch. Again no effect was obtained, so that a stimulus due to volatile substances does not account for the reaction. It remained to show that the stimulus was due to a chemical substance transmitted through the medium or that organic contact between the two mycelia was necessary. A thin layer of medium was poured over a sterilized slide and cut into two parts by removing a strip 4–5 mm. wide from the centre. A culture of 16,1 was placed on one side of the strip and one of 16 on the other and the slides were then incubated for forty-eight hours. On examination under the microscope it was found in some of the slides that hyphae of the two strains had met and fused with one another (hyphal fusion is common in this fungus even between hyphae of the same mycelium) while at the same time the hyphae of one strain had not yet reached the medium in which the other was growing. Inocula of 16,1 were taken from the edge of the medium. These were grown for some days, when about 50 per cent. were found to have formed fluffy colonies while the remainder were normal. It was observed, however, that the fluffy type of growth was very quickly replaced by the thin, normal type. Numerous inoculations were made from slides where the two sets of hyphae, while close to one another, had not yet met, but in no case was a fluffy colony obtained. It is concluded that organic contact is probably necessary before the alteration in the type of growth of 16,1 can occur, but that the effect is of a transitory nature only. It is realized that the procedure adopted does not rule out the possibility of the effect being transmitted along the moist surface of the slide between the two cultures, but as the effect was only observed where organic contact had been established and not otherwise it is thought unlikely that this does in effect take place.

#### IV. SEGREGANTS OF A CROSS BETWEEN A NORMAL AND A MUTANT HAPLOID.

The mutant strain A had been kept for three years in culture before it was paired with different haploids of the new stock of *C. macrorrhizus*. It was found in every case, irrespective of the sex of the second strain, that a diploid colony was formed. It was concluded from this that the two stocks, obtained at different times and from widely separated localities, differed in their sex factors so that those of one stock were allelomorphic to those of the second. This result is similar to that commonly obtained with *C. lagopus*.

The diploid produced by pairing strain A (Pl. XII, Fig. 41) old stock, with strain 14, new stock, was grown on sterilized dung, and gave rise to fertile fruit bodies. Spores from one of these were sown, and single-spore colonies grown and compared for growth-rate and appearance. 80–90 per cent. of the spores germinated, but, of the sporelings produced, 50 per cent. had only a limited amount of growth, and were similar to the dwarfs

obtained in all crosses of *C. sphaerosporus* (2 and 3). Fifteen morphologically distinct types were found among the segregants. The frequency distributions of the growth-rates of the members of each type are shown in Text-fig. 2, and photographs of each type in Pl. XII, Figs. 25-39. The



TEXT-FIG. 2. For explanation see text.

growth-rates ranged from 14 to 40 mm. in four days. The two types, numbers 1 and 31, with greater rates of spread than the faster parent, were very thin. Types 30 and 45 were respectively identical with the two parental strains 14 and A in appearance (cf. Pl. XII, Figs. 38, 39, 41 and 40), and similar in growth-rate. The various strains differed morphologically in a marked degree. All were colourless like their parents with the exception of types 1, 43, and 44, where there was a certain amount of lemon yellow-coloration, though whether this was present in the hyphae or in the substrate was not determined. The relative frequencies of occurrence of the various types are shown in Text-fig. 2. It will be seen that each of the non-parental types, numbers 1, 10, 19, 27, 31, and 43, occurred with greater frequency than either one or both of the parental types 30 and 45. This result is similar to that obtained in most crosses of *C. sphaerosporus*.



## V. SEGREGANTS FROM 'ILLEGITIMATELY' PRODUCED DIPLOIDS.

Buller (1) found that, in the case of *C. lagopus*, not only could diploids 'legitimately' diploidize haploids as when the haploid was of the same sex as one or other of the haploids which formed the diploid (e.g.  $AB/AB \times ab$ ), but that 'illegitimate' diploidization was possible in which the haploid was of a different sex from that of either of the haploids from which the diploid arose (e.g.  $AB/Ab \times aB$ ). These experiments have been repeated, using the new stock of *C. macrorhizus* with the following results.

Two diploids  $19 \times 11$  ( $AB \times ab$ ) and  $16 \times 6$  ( $aB \times Ab$ ) were paired respectively with seven and nine haploids (including in each case strains 16 and 16,1). In all cases the haploids were diploidized. Of those paired with  $19 \times 11$ , four were illegitimate and three legitimate, and of those with  $16 \times 6$ , three were illegitimate and six legitimate diploidizations. In some cases diploidization took place more slowly than in others, but this applied equally to the legitimate and illegitimate matings. The saltant 16,1 behaved similarly to 16. There was considerable variation as to the manner in which the haploid became diploid. In some cases patches of diploid mycelium occurred at intervals along its free periphery (vide  $1/19 \times 11$ ,  $AB/AB \times ab$ , Pl. XII, Fig. 23), which grew rapidly and soon met one another. In other cases the haploid was diploidized at all points on its periphery at about the same time, e.g.  $16\ 1/19 \times 11$ ,  $aB/AB \times ab$ , Pl. XI, Fig. 22). Fig. 21,  $16/19 \times 11$ , shows a type intermediate between these two. Fig. 24 ( $16,1/16 \times 6$ ,  $aB/aB \times Ab$ ) shows a third type in which diploidization began at one side of the free periphery, and spread regularly around the haploid. From repeated pairings it has been found that these types are not in any way typical of a particular combination of haploids or sexes, but rather are examples of the range of reactions which may be obtained in any single combination of strains.

Three haploids diploidized by diploids were selected for breeding purposes, namely,  $21/19 \times 11$  ( $Ab/AB \times ab$ ),  $16,1/16 \times 6$  ( $aB/aB \times Ab$ ) and  $16,1/19 \times 11$  ( $aB/AB \times ab$ ). The first and third are illegitimate, and the second a legitimate mating. 150 single-spore colonies were selected at random from spores of each strain, and subcultured, five together, in a Petri dish. Practically all the spores germinated in each case. Table I shows the percentage of colonies transferred which grew to macroscopic size (some of those subcultured were dwarfs, and perhaps 5 per cent. failed due to their slipping off the needle, mechanical injury, or other similar cause), and of these the numbers of abnormal or mutant types.

Six Petri dishes representing thirty transfers from each strain are shown in Pl. XII, Figs. 42, 43, and 44. Fig. 42 shows colonies from the diploid  $21/19 \times 11$ . One inoculum has not given rise to a colony. It will be seen that all the segregants are very similar (minor differences in size

being accounted for by differences in size of inocula), but there are present a number of the normally recurring mutant types with a slow growth-rate and dense mycelium. The six plates were selected to show these types. Figs. 43 and 44 are of segregants from the diploids  $16,1/19 \times 11$  and  $16,1/16 \times 6$  respectively. In each case a number of mutant forms are seen. These are of more than one type, variation being greater in  $16,1/16 \times 6$  segregants than in those of  $16,1/19 \times 11$ . The range of types is much smaller in these crosses than in the cross  $A \times 14$ .

TABLE I.

Strain.	Macroscopic colonies.	Abnormals.
	%	%
$21/19 \times 11$ , illeg.	94	6 (normal mutants)
$16,1/16 \times 6$ , leg.	88	27
$16,1/19 \times 11$ , illeg.	98	35

Fifteen strains from  $21/19 \times 11$  were selected at random and respectively examined for sex by pairing with four tester strains. Eight mutant and four normal segregants from  $16,1/19 \times 11$  were similarly treated. The results of the two tests are shown in Table II.

TABLE II.

Sex group.	Segregants of $21/19 \times 11$ .				Segregants of $16,1/19 \times 11$ .			
					Mutants.		Normals.	
ab	+	-	-	-	+	-	+	-
aB	-	+	-	-	-	+	-	-
Ab	-	-	+	-	-	+	-	+
AB	-	-	-	+	-	-	-	+
Frequency	1	2	7	5	0	6	1	1

The plus sign indicates that a strain belongs to the sex group indicated, the negative that there was no indication that it belonged to that particular group. No segregant reacted positively with more than one tester strain. It will be seen that among the segregants of  $21/19 \times 11$  those of sex Ab had the greatest frequency and strain 21 is of sex Ab. Among the mutants of  $16,1/19 \times 11$ , sex aB occurs with the highest frequency and 16,1 is of this sex.

## VI. DISCUSSION.

The observation that one of the four groups of *C. macrorhizus* produces a type of saltant sector different from those common to the other three sex groups, indicates that it may be possible by means of X-rays to distinguish the strains of one sex group of the four without having resort to mating tests. As the old and new stocks of *C. macrorhizus* were allelomorphic to one another as regards each pair of sex factors it appears that the factor conditioning the peculiar type of saltant is distinct from either of the sex

factors. As only twelve haploids have been X-rayed it is not possible to say definitely that a particular sex is always associated with this reaction, but should this prove to be so it would indicate a close linkage between one or other sex factor and the factor responsible for the appearance of the peculiar saltant following irradiation.

On pairing strains of this saltant type with normal strains of all four sexes they were found to be diploidized by strains of opposite sex to their parent. The other three strains acted on the saltant in a somewhat similar manner, though in some cases strains of the same sex as the saltant produced a slightly different reaction from that found where the remaining two sexes were concerned. It was never possible, however, definitely to distinguish the three sexes from one another by their reactions with the saltant.

As it has been shown that the stimulus of a parental strain in causing increased mycelial production in one of these saltants is not transmitted through glass or quartz or across a short air-gap it follows that either the effect is conveyed through the medium or else that organic contact between the two reacting strains is necessary. Experiment indicates that the latter is almost certainly the case. As the effect on the saltant is a transitory one it is unlikely that a transference of nuclei from the normal to the mutant strain occurs. The stimulus traverses the saltant mycelium in exactly the same way as the phenomenon of diploidization, namely, primarily round the periphery, starting from the point of contact of the reacting strains, and secondarily centripetally to a slight extent. The two reactions are also alike in that the effect is transmitted at a faster rate than the mycelium spreads. In old cultures the range of influence of the normal on the mutant strain decreases. Whereas in a young culture the saltant is affected at all points on its periphery, in an old culture only the edge of the saltant adjacent to the normal strain is affected. It is more probable that the lack of response on the part of an old saltant colony is due to its incapacity to respond than that the normal strain loses the power of stimulating the saltant.

Segregants from a cross between a normal and a saltant strain display a similar range of types to those obtained in crosses between the naturally occurring morphologically heterogeneous strains of *C. sphaerosporus*. Their phenotypes and rates of spread bear a similar relationship to those of the normal strains that the numerous haploid types of *C. sphaerosporus* bear to the type *e* haploids. This similarity has been advanced in support of the suggestion that type *e* might be regarded as the 'wild type' of *C. sphaerosporus* from which the other phenotypes were deprived (3). As the effect of X-rays is not infrequently to cause chromosome abnormalities it is possible that aberrations of one form or another are responsible for the numerous phenotypes obtained in this cross. If, on the other hand, the effect had been purely one of changing individual genes, then at least four

genes must have mutated to account for the production of the fifteen segregating types. Another analogy may be drawn between the reactions of the two *Coprinus* species, namely, the production of dwarfs. These have been found to occur in all crosses of *C. sphaerosporus* (a cross between two type *e* strains was not made), but in *C. macrorhizus* they are only found among the segregants of a cross containing a mutant strain and never among the progeny of a diploid produced from two normal haploids.

While Buller's experiments on *C. lagopus* have not been repeated, it is evident from a comparison of his results with those described here that illegitimate diploidization takes place with greater readiness in *C. macrorhizus* than it does in *C. lagopus*. In the former fungus all attempts to induce diploidization in a haploid by pairing it with either a legitimate or an illegitimate diploid were successful, and the resulting strains where tested proved fertile in every instance.

Two theories have been advanced to explain the illegitimate combinations demonstrated by Buller (1), working with *C. lagopus*. One of these was put forward by Quintanilha (5), in which he suggests the occurrence of an abnormal nuclear division in the vegetative mycelium, during which nuclei of a dicaryon exchange relevant chromosomes thereby enabling a legitimate diploidization to take place. The second was advanced by Rawitscher (6), in which it is supposed that in an illegitimate combination such as  $AB/Ab \times aB$  the haploid mycelium receives both the nuclei of the diploid  $Ab \times aB$ , and that the latter divide conjugately and displace the original  $AB$  nuclei. It has been shown by two distinct methods (2 and 3) that in the case of legitimate combinations of *C. sphaerosporus* (as the species is bipolar it is impossible to produce illegitimate pairings) that the nuclei of the haploid are not pushed aside but play a major part in subsequent basidiospore formation, and one of the methods also indicates the survival of the original diploid dicaryon which takes part in spore production. It is obvious from the experiments described in Section V, in which it was shown that mutant types which could only have been derived from nuclei of the haploid appeared among the segregants of an illegitimate combination ( $16,1/19 \times 11$ ) that Rawitscher's theory does not apply in this case. The following theory is based on *a*, the results of sex tests, *b*, the appearance of mutant types among the segregants and *c*, the percentage of dwarfs among the sporelings. In a legitimate combination such as  $16,1/16 \times 6$  nuclei of  $16,1$  combine with those of strain 6 to form dicaryons which ultimately give rise to basidiospores and at the same time the original ( $16,6$ ) dicaryons enter the haploid mycelium and also give rise to spores, though the number of ( $16,6$ ) dicaryons may be less than the number of ( $16,1, 6$ ). In the case of an illegitimate combination, e.g.  $16,1/19 \times 11$ , it is suggested that ( $19,11$ ) dicaryons enter the haploid in sufficient numbers to cause it to assume the diploid form and to instigate the production of

apparently normal diploid fruit-bodies, and at the same time the nuclei of the haploid (16,1) divide and give rise parthenogenetically to basidiospore tetrads as is the case in haploid fruit-bodies of *C. lagopus*. (It was not determined whether haploids of *macrorrhizus* would produce haploid fructifications under suitable conditions, but from analogy with *lagopus* and taking into account the close similarity which exists between the two forms it is to be expected that they would do so). Thus, in an illegitimate combination the mycelium would be a nuclear chimaera partly normal diploid and partly normal haploid. A somewhat similar chimaeric theory has been advanced by Oort (4) using *C. lagopus*. He found that  $AB \times aB$ ,  $Ab \times ab$ ,  $AB \times Ab$ , and  $aB \times ab$  combinations all gave rise to spores of two sexes—those of the parental strains—and suggested the production of a type of haploid chimaera to account for this result. In the experiments on *macrorrhizus* four sexes have been found segregating from each combination. This was also found to be the case by Buller (1) and Quintanilha (5) using illegitimate combinations of *C. lagopus*, but the latter does not record any difference in the relative frequencies of occurrence of the sexes. In the legitimate case of  $16,1/16 \times 6$  it is evident that the 16,1 nuclei play a part in spore formation as mutant types are present in 27 per cent. of the (macroscopic) offspring. That 16,1 nuclei go through a meiotic division is probable from the occurrence of different types of mutant among the segregants, and this is also indicated by the presence of 12 per cent. of transferred spores which did not reach macroscopic size, and of which some were in all probability dwarfs. It is not possible from the data available to show that (16,6) dicaryons actually gave rise to spores, but from the homologous case of *C. sphaerosporus* this is to be expected. The presence of 35 per cent. mutant types in the segregants of the illegitimate cross  $16,1/19 \times 11$  indicates definitely that the nuclei of 16,1 give rise to spores as in the legitimate combination. Since, however, as previously stated, the variation is less in the case of the illegitimate than in that of the legitimate combination, it is possible that such variation as was found could be adequately accounted for, either on the grounds of continuous variation for the minor differences and of an occasional mutation for the 1 or 2 per cent. of more distinctly different types, without the assumption of meiotic segregation. Occasional mutations are known to occur in normal strains, and would be expected to occur with greater frequency among the saltants if, as is probable, they have arisen as a result of chromosome abnormalities induced by X-rays which would involve loss and abnormal separation of chromosome fragments in successive mitoses. The presence of only 2 per cent. of non-macroscopic colonies, indicating the complete absence of dwarfs which both here and in *C. sphaerosporus* have always been found following meiotic segregation in a normal  $\times$  mutant diploid, also lends weight to this hypothesis. A greater number of abnormals (i.e. mutant types) in segregants from the illegitimate

than in those of the legitimate combinations would be expected on this theory, and this has been found by experiment to be the case, the ratio being 35:27. For while every (16,1) nucleus in the illegitimate mating will give rise mitotically to abnormal sporelings, each (16,1) nucleus of the legitimate combination must first pair with a normal (6) nucleus and then undergo reduction division, as a result of which it is to be expected that one or more spores of the tetrad will give rise to colonies having a normal phenotype.

Turning to the interpretation of the sex tests it is seen that segregants of the same sex as that of the haploids in each of the illegitimate matings (apart from the normals of  $16,1/19 \times 11$ ) are more numerous than those belonging to any of the other sexes. This would not be the case if a normal meiotic division were present. In the case of the normal segregants of  $16,1/19 \times 11$  there is no such predominance of one particular sex, and this also supports the hypothesis as the normal strains are presumed to have undergone a normal reduction. Buller (1, p. 251), in a similar experiment with *C. lagopus*, found the sexes from a combination  $Ab/AB \times ab$  to occur in the ratio of 3 AB:3 ab:2 Ab:1 aB:1 a'b' (the latter strain being perhaps a mutant). As he only determined the sex of ten spores the absence of an excess of spores of sex Ab does not invalidate the findings recorded above. Also, if as suggested, the mycelium from such a combination is a chimera, it is to be expected that the relative numbers of single nuclei and dicaryons will vary from one hypha to another. That mutants, one of sex Ab and the other of sex AB, should occur among the segregants of  $16,1/19 \times 11$  is not to be expected from the theory outlined above; but if as previously suggested, mutation of normals does occasionally occur, an inclusion of such a mutant among those derived from 16,1 would account for the discrepancy. In view of this explanation, and taking into consideration the possibility of experimental error, it is held that, considering the evidence of the rest of the data, the presence of these two sex strains among the mutants does not invalidate the hypothesis outlined above.

It should be possible to determine the correctness of the hypothesis set out here on more extensive investigation being undertaken along similar lines. The mutant types of  $16,1/16 \times 6$ , for example, should, if meiosis is involved, belong to all four sex groups. An examination of spore tetrads would also be of very definite value. In the case of a legitimate combination (e.g.  $16,1/16 \times 6$ ) some tetrads should give rise to one or more mutant forms, i.e. those from (16,1, 6) dicaryons, and others from (16,6) should produce only normal strains. Not infrequently the abnormal strains from a single tetrad should be phenotypically different and at the same time all tetrads should contain the four sex groups. In an illegitimate combination such as  $16,1/19 \times 11$ , mutant forms should all be of the same sex, and should exhibit similar phenotypes in all tetrads (allowance being made for an

occasional mutation). Normal strains should be of all four sexes in approximately equal numbers.

#### VII. SUMMARY.

Irradiation by X-rays has induced a peculiar type of saltant sector in several haploid strains of *Coprinus macrorhizus*. These strains were all of one sex, and it is possible that this saltant type can only be produced in haploids of this sex. At the same time the factor conditioning this particular response is distinct from either of the sex factors.

The reactions of the saltant with its parent and other normal strains are described, and experiments indicate that a necessary preliminary to the production of these reactions is organic contact between the two strains. As the effect is transitory it is improbable that a transference of nuclei from one mycelium to the other takes place.

Segregants from a cross between a normal and a mutant strain are described, and their growth-rates determined. The results are shown to be similar in all respects to those obtained with *C. sphaerosporus*.

It is shown that in this fungus legitimate and illegitimate combinations are produced with equal readiness. Segregants from two illegitimate and one legitimate combination were grown and compared, and the sex of certain strains of the two illegitimate combinations determined. These experiments have shown that the nuclei of the haploid play a major role in spore formation. A theory supported by the experimental evidence is advanced in which it is supposed that in a legitimate combination the mycelium is a nuclear chimaera, in that it consists of a mixture of two sets of dicaryons; whereas in an illegitimate combination, while the mycelium is again chimaeric and contains two nuclear sets, one of these is a haploid and the other a diploid set.

These experiments were begun in 1931 at the John Innes Horticultural Institution, and I have much pleasure in recording my gratitude to Sir Daniel Hall for affording me facilities for work.

My thanks are also due to Professor V. H. Blackman for his encouragement and valued criticism during the latter part of the investigation.

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## DESCRIPTION OF PLATES XI and XII

Illustrating the paper by Dr. H. Dickson entitled 'Observations on Inheritance in *Coprinus macrorhizus* (Pers.) Rea'.

## PLATE XI

All the figures, with the exception of 9, 26, 37, 38, and 41 have been photographed by reflected light. In Fig. 9 both transmitted and lateral light were used, and in the Figs. 26, 37, 38, and 41 both reflected and transmitted, as it was found that the colonies were too thin to be photographed by reflected light only. All photographs, with the exception of Fig. 3, are reproduced on approximately the same scale. Fig. 3 was grown on a 14 cm. Petri dish and is much reduced. Strain A is the uppermost colony in Figs. 10-14, and strain 16,1 in Figs. 15-20 and 22.

Figs. 1 and 2. Common sector types following irradiation in a strain of sex AB (old stock).

Figs. 3-9. Peculiar sectors following irradiation in strains of sex aB. Fig. 3 shows the origin of saltants A and B. At the periphery of the A sector a very thin mycelium can be seen. Figs. 8 and 9 are of the same culture. In Fig. 9 the well-marked ridge where the saltant and parent meet can be seen.

Figs. 10-14. Various stages showing the development of the fluffy aerial mycelium in strain A (thin colony) after making contact with its parent strain. The dark sector in Fig. 14 is probably a saltant.

Figs. 15-17. The saltant 16,1 paired with 16. Note the straight line of junction between the two strains in Fig. 17.

Figs. 18 and 19. Two stages of the reaction between 16,1 (sex aB) and 19 (sex AB).

Fig. 20. Strain 16,1 diploidized by strain 21 (sex Ab).

Figs. 21 and 22. Two illegitimate combinations 16,1/19  $\times$  11 and 16,1/19  $\times$  11 respectively. Strains 16 (uppermost) and 16,1 have each been diploidized.

## PLATE XII

Figs. 23 and 24. Two legitimate combinations, 1/19  $\times$  11 (AB/AB  $\times$  ab) and 16,1/16  $\times$  6 (aB/aB  $\times$  Ab) respectively. Strains 1 and 16,1 are uppermost and have been diploidized in each case.

Figs. 25-39. Segregating types from the cross A  $\times$  14. Respective type numbers 41, 44, 25, 22, 42, 27, 48, 38, 10, 31, 19, 43, 1, 45, 30.

Fig. 40. Strain 14.

Fig. 41. Strain A.

Fig. 42. Segregants from the illegitimate combination 21/19  $\times$  11 (Ab/AB  $\times$  ab).

Fig. 43. Segregants from the illegitimate combination 16,1/19  $\times$  11 (aB/AB  $\times$  ab).

Fig. 44. Segregants from the legitimate combination 16,1/16  $\times$  6 (aB/aB  $\times$  Ab).





# A Study of Heart-rot of Young Sugar-beet Plants Grown in Culture Solutions.

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With eleven Figures in the Text.

## INTRODUCTION.

WARINGTON (10) has reported on the changes in anatomical structure induced in broad beans by growing them in a medium free from boron. She found that wilting and ultimate death of the plants was preceded by basipetal degeneration of the tissues from the apex, which was at first confined to the cambium and vascular tissues of the plant. In *Vicia faba* Warington found that, if the plant was not irreparably damaged, the addition of boron to the nutrient solution caused the development of new axillary shoots at the base of the main shoot.

It has been known for some time that the disease called 'heart-rot' in sugar-beet is due to boron-deficiency. This fact has been demonstrated by Brandenburg (2, 3), de Haan (4), Kaufmann (7), Foex and Burgevin (6), Bobko and Belousov (1), &c. In view of the abnormal type of secondary thickening in Beta it was thought that a study of the anatomy of boron-starved plants might prove interesting since, normally, separate concentric cambia are cut off in the ground tissue at intervals throughout the life of the plant. It seemed possible that the death of one cambium need not necessarily preclude further growth in thickness in the same organ when boron was restored to the nutrient solution. Thus, zones of normal and degenerating tissue might be obtained in the same organ, and an opportunity would be provided for observing stages in disintegration.

Reference to the literature made it clear that sugar-beet is very sensitive to boron in great dilution, and that just as in broad bean, while a concentration of 1 : 1,000,000 boric acid is enough for normal development, in the absence of boron growth is inhibited and disintegration sets in. Accordingly, a number of sugar-beet seedlings were raised and transferred to culture solutions.

## EXPERIMENTAL METHODS.

Fruits of sugar-beet were set in damp sawdust and seedlings were available in about nine days. Forty-two of these were transferred to covered bottles containing nutrient solution of the following constitution :

KNO <sub>3</sub>	1 g.	KH <sub>2</sub> PO <sub>4</sub>	0.3 g.
MgSO <sub>4</sub>	0.5 g.	K <sub>2</sub> HPO <sub>4</sub>	0.27 g.
CaSO <sub>4</sub>	0.5 g.	Fe <sub>2</sub> Cl <sub>6</sub>	0.04 g.
NaCl	0.5 g.	MnSO <sub>4</sub>	0.001 g.

Water to make up to one litre.

This is one of the Rothamsted food solutions, and its pH is about 6.3. The chemicals used had previously been tested by spectroscopic methods, and were guaranteed to be free from all traces of boron.

The experiment was begun in the second week in May 1935, and terminated by fixation of the material on 6th August of the same year.

PLAN OF THE EXPERIMENT. TABLE I.

(Seeds set in damp sawdust on 9th May 1935.)

Set.	16th May.	5th June.	26th June.	15th July.	31st July.	6th Aug.
A	—B	B	B	B	—B	Fixed
B	—B	2B	2B	2B	—B	„
C	—B	—B	—B		dead	
D	B	B	—B	+B	B	Fixed
E	B	B	B	—B	—B	„
F	B	2B	2B	4B	8B	„

As a fixative, 2BE (LaCour(8)) was used, and this gave good results. After sectioning the material, it was found that staining with gentian violet alone was more satisfactory than the use of a double stain.

The plants were divided into six sets of seven each (see Table I).

I. *Plants grown without boron for the first three weeks.*

*A. Transferred to a solution containing 1 p.p.m. H<sub>3</sub>BO<sub>3</sub> on 5th June, and deprived of boron again one week before fixation.*

These plants were greatly retarded at the end of the first three weeks, most of them having small root-systems and no expanded leaves except for the cotyledons. After the transference to boron, however, the growth-rate rose considerably, although at six weeks old the hypocotyls were not yet swollen, and the leaves were still very small as compared with the sets that from the outset had been supplied with boron. Recovery continued in all except one plant, which succumbed in the tenth week. The rest of the set had several expanding leaves and swollen hypocotyls by the end of July. On 31st July they were replaced in boron-free solutions, but no signs

of heart-rot, either external or internal, had set in when they were fixed on 6th August.

*B. Transferred to a solution containing 2 p.p.m.  $H_3BO_3$  on 5th June, and deprived of boron again one week before fixation.*

Recovery after the initial three weeks deprivation of boron was more rapid in this set than in that receiving 1 p.p.m. of boric acid (the A set), and none of the plants died. By the middle of July all of these were sturdy plants with swollen hypocotyls, and growth in boron-free solutions for a week before fixation produced no sign of heart-rot.

In both the A and the B sets the original terminal bud had died, and growth was continued by two or three closely-set axillary buds at the top of the beets.

*C. Maintained in boron-free solutions.*

These plants did not grow beyond the seedling stage, and by 28th June they were all dead.

## II. *Plants grown with 1 p.p.m. boric acid for the first three weeks.*

*D. Transferred to fresh solutions containing boron for three weeks, deprived of boron on 26th June for a further three weeks, then supplied with boron until the end of the experiment three weeks later.*

These plants grew well, and had a number of leaves and thickened hypocotyls by 26th June. A fortnight after this all of these plants showed unmistakable signs of the disease. The older leaves were quite healthy, but the bases of the younger leaves and the apical buds were black and shrivelled. With the restoration of boron on 15th July these plants began to recover. By 31st July, all the blackened leaves had dried up, and new green leaves from axillary buds and new roots were developing rapidly, progress continuing steadily until the end of the experiment. The plants appeared rather bunchy owing to the large number of small leaves growing out from the top of the hypocotyl. On cutting the swollen beets open longitudinally, it was seen that they were all more or less marked with brown streaks.

*E. Transferred to fresh solutions containing 1 p.p.m.  $H_3BO_3$  for another six weeks, deprived of boron on 15th July, and kept without boron until the end of the experiment.*

By 15th July these beets were considerably swollen, and each had a number of well-grown leaves. After a fortnight in boron-free solutions blackening of the apical buds and the bases of the younger leaves had taken place, while the older leaves remained quite healthy in appearance. This condition became accentuated during the last week of the experiment,

gradually including older leaves as the disease spread outwards. The beets were split open longitudinally, and were all found to be more or less marked with brown streaks at the top, which were particularly evident in one plant.

F. Given 2 p.p.m.  $H_3BO_3$  on 5th June, 4 p.p.m. on 15th July, and 8 p.p.m. on 31st July.

Throughout the experiment, these plants were healthy and actively growing. After six weeks in solutions containing 2 p.p.m.  $H_3BO_3$ , they showed no improvement on the last (E) set that had, up to that time, been supplied with 1 p.p.m. The higher concentrations given later on had no apparent adverse effect on the metabolism, as it was clear that the plants had not been poisoned by a concentration of 8 p.p.m.  $H_3BO_3$ .

Considerable resistance to the absence of boron in the nutrient solution was shown by the young seedlings of sets A, B, and C, and this was presumably due to the presence of this element in the cotyledons. Before the end of three weeks this reserve was exhausted, and doubtless the plants would not have recovered if the addition of boron had been delayed much longer. The plants were only thirteen weeks old when they were fixed, and through the whole of this time boron appeared to be of definite importance in the metabolism of this plant, since at all stages, after that of the young seedlings, signs of disintegration were visible in ten days after the change-over to a boron-free solution, although not in less time than this.

## STRUCTURAL CHANGES DUE TO ABSENCE OF BORON.

### I. *General outline.*

A study of serial sections of a beet reveals the fact that each of the rings of secondary vascular tissue, to the development of which the swelling of the beet is due, is composed of downward continuations of leaf-traces. These vascular rings are considered by Eames and MacDaniels (5) to originate in the proliferating pericycle. The rings are not regular, but consist of two or more arcs, each representing a single leaf-trace. Probably the number of arcs in each ring depends on the phyllotaxis of the leaves to which they are connected.

### *Sets A and B.*

(Grown without boron for three weeks, then given boron till a week before fixation).

In these plants, as a result of the early destruction of the original growing point due to a lack of boron, two or more buds have grown out, each giving rise to its own set of vascular rings. The diseased vascular system has been pushed over to one side, and appears in transverse view near the top of a longitudinal section of the beet (Fig. 1).

*Set D.*

(Grown in the presence of boron except for three weeks during June and July).

In this set of beet the central vascular ring is without any sign of cellular disintegration. The next few rings are normally developed, but

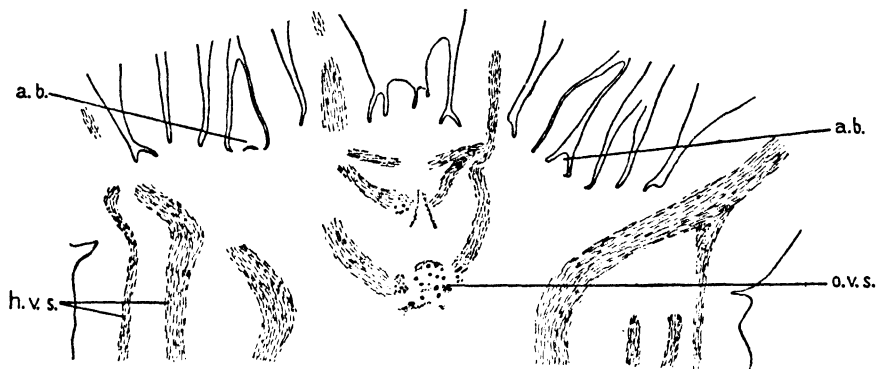


FIG. 1. Nearly median longitudinal section of a beet from the A set (grown without boron for three weeks, then given boron until a week before fixation). *o. v. s.* original vascular system of the seedling cut transversely. Diseased cells are dotted. The scattered cells represented are vessels. *h. v. s.* healthy vascular strands formed by the leaves which were developed on recovery of the plant. *a. b.* axillary buds which have continued the growth of the beet since the destruction of the original terminal bud.  $\times 25$ .

frequently disintegration of the parenchyma immediately inside the xylem has occurred, and in some places the phloem shows signs of the disease, either by hypertrophy of the cells, or by the blocking of most of the sieve-tubes. The fifth ring in Fig. 2 is represented by disintegrated or abnormal cells in groups. Evidently the cambium had not long been initiated here when the consequences of boron deficiency became effective. It appears that before disintegration set in, differentiation was accelerated, and vacuolization, swelling, the development of intercellular spaces, and the thickening of the cell walls took place. Some of the surrounding parenchyma cells became hypertrophied and cross divided by extremely thin walls, while other cells became crushed (Fig. 4). Immediately outside these patches of disintegrated cells is a ring of healthy, normally developed vascular tissue (Figs. 2 and 3), succeeded by two rings of cambium. The cells that have been cut off from the latter have not yet become differentiated. Evidently these last three rings are connected with the new leaves developed from the top of the beet after the restoration of boron to the nutritive solution.

*Set E.*

(Grown with boron until three weeks before fixation).

These plants spent the last three weeks in boron-free solutions. As in Set D, the inner secondary vascular zones show very little heart-rot, while

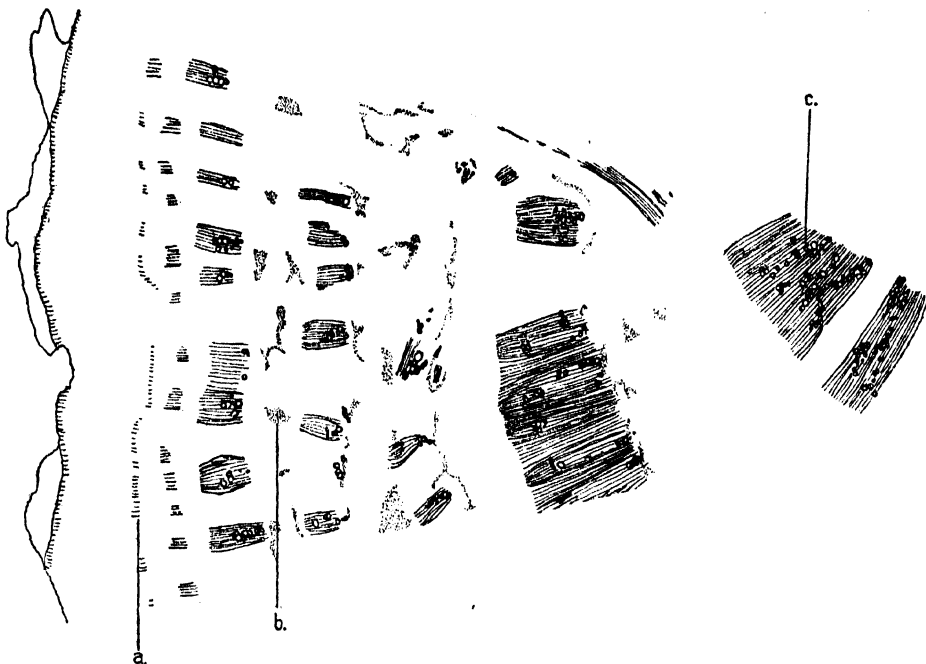


FIG. 2. Diagram of the vascular arcs in a sector of a transverse section taken some distance below the apex, from a beet of the D set (grown in the presence of boron except for three weeks in June and July). Healthy vascular zones shown by close parallel lines, with the vessels in outline. Diseased vascular tissue and parenchyma dotted. *a*. Youngest zone of secondary vascular tissue-cells as yet undifferentiated. *b*. Zone of diseased vascular tissue that must have been in process of differentiating when the disease set in. *c*. Oldest vascular ring—still quite healthy.  $\times 45$ .

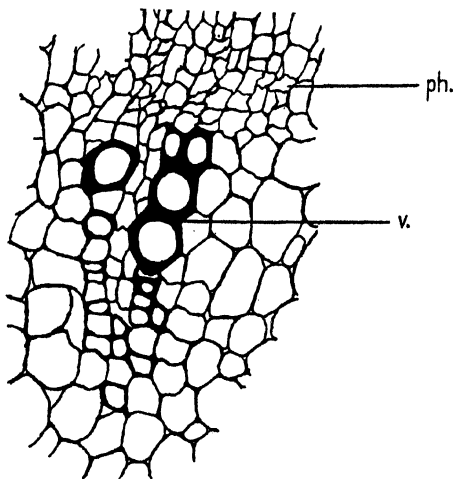


FIG. 3. A vascular strand from the arc immediately outside the diseased vascular ring shown in Fig. 2. *ph.* Phloem. *v.* Vessels.  $\times 450$ .

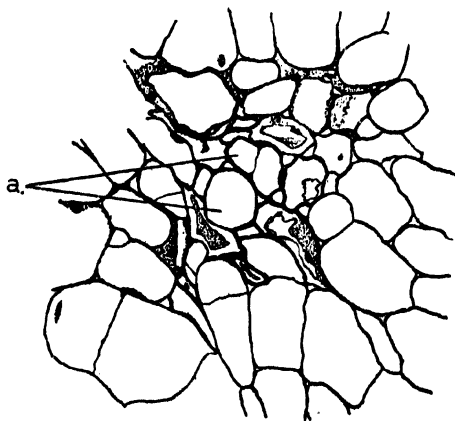


FIG. 4. Strand from the diseased vascular zone in Fig. 2. *a*. Cells which in normal conditions would have become vessels.  $\times 450$ .

the parenchyma adjacent to the younger vascular arcs often exhibits stages in disintegration. The outermost arc of meristematic cells, representing the activity of a new cambium, consists of an incomplete zone, several cells in width. This is diseased, the cells being crushed between hypertrophied



FIG. 5. Diagram of the vascular arcs in a sector of a transverse section taken some distance below the apex, from a beet of the E set (grown in the presence of boron until three weeks before fixation). Healthy vascular zones shown by close parallel lines, with the vessels represented in outline. Diseased vascular tissue and parenchyma dotted. *a*. Outermost arc of cambium—diseased. *b*. Innermost vascular ring—healthy. The intermediate rings are all slightly diseased.  $\times 45$ .

cortical cells (Figs. 5 and 6). In longitudinal section (Fig. 7), the youngest leaf-trace is seen immediately below the leaf bases, on its outward course from the growing point of the beet to the periphery.

#### Set F.

(Grown with boron throughout, increasing in quantity with the age of the plant).

At the close of the experiment, the axes of these plants had attained to a maximum diameter of one inch, growth of the secondary vascular rings being normal throughout the axis.

## II. Anatomical details.

The meristematic tissues of the shoot are the first to degenerate as a result of boron deficiency. The apical growing point of the stem, the embryonic leaves, and the newly developed cambia rapidly disintegrate. The cells of the vascular system that were in process of differentiating when

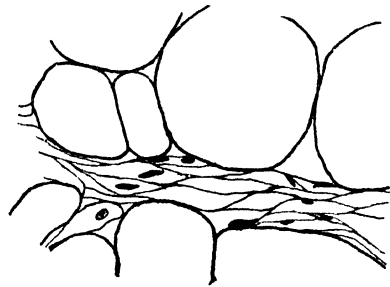


FIG. 6. Part of the crushed cambium of the outermost leaf-trace from Fig. 5.  $\times 990$ .



boron was withheld appear also to be very susceptible to the omission. Frequently the cells become thick-walled but apparently not rigid, since hypertrophy of the surrounding cortical cells causes them to assume very irregular shapes (Fig. 4). Many of the cells of the ground tissue and a few



FIG. 7. Nearly median longitudinal section of a beet from set E (grown with boron until three weeks before fixation). *d.t.* entirely disintegrated tissue, including the bases of the youngest leaves and the tissue round the actual apex, which are shaded in black. *d.v.s.* diseased vascular strands, marked in thick lines. *h.v.s.* healthy vascular strands of the outer leaves and of the beet, shaded in thin lines when cut longitudinally and in dots representing the vessels when cut transversely.  $\times 25$ .

of those in the diseased vascular strands appear to have undergone division, and the two daughter cells are separated by an extremely thin wall. In the later stages of the disease, where older vascular arcs are affected, the cambial cells have become irregularly swollen and divided, and much of the phloem and parenchyma is completely disintegrated (Fig. 8).

Not infrequently the sieve-tubes of healthy plants contain plugs of densely staining material which presumably is callose. This is sometimes developed as a solid rod throughout the cell (Fig. 9), or disposed as a peripheral lining. These phenomena occur much more frequently in the phloem of diseased plants, where they are very typical. It may be that their development is the characteristic precursor of the subsequent degeneration in the mature tissues of vascular strands, since they are sometimes frequent in strands which do not exhibit any other signs of disease.

Root-tips of diseased plants were examined, and these showed features similar to those observed by Sommer and Sorokin (9) in boron-starved peas, viz. differentiated vascular tissue occurred much closer to the apex than in healthy roots, and none of the nuclei were dividing. It would appear either that there is some difference between the growing points of root and shoot, since the root-tip does not degenerate at the same time as the shoot apex,

or that the minimum amount of boron necessary for the mere maintenance of the root-tip is retained within its cells.

The cambial arcs arise centrifugally in the beet, and the peripheral ones, being the youngest, are therefore connected with the innermost

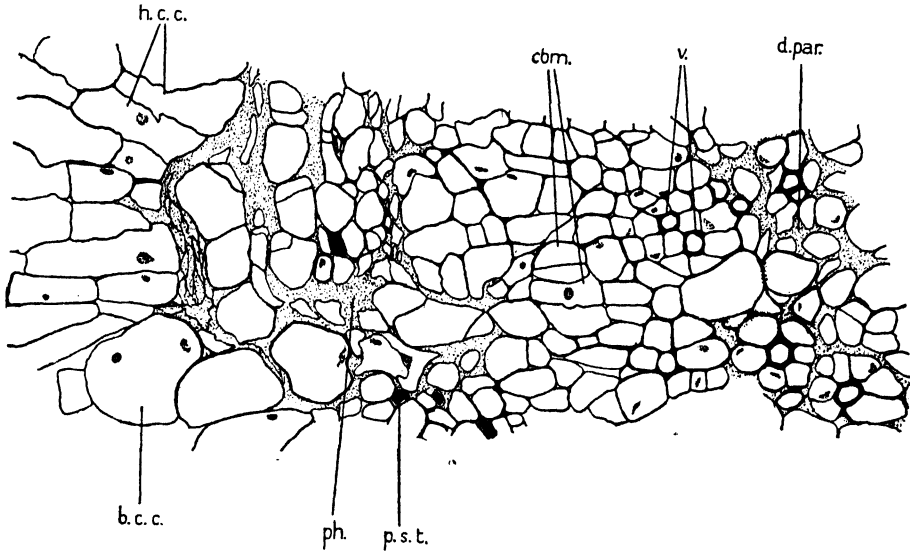


FIG. 8. Transverse section of a vascular strand from near the apex of a plant that had been deprived of boron for three weeks. *ph.* disintegrated phloem. *d.par.* disintegrated parenchyma. *p.s.t.* plugged sieve-tube. *b.c.c.* binucleate cortical cell. *v.* vessels. *h.c.c.* hypertrophied cortical cells. *cbm.* irregular and hypertrophied cells of the cambium.  $\times 450$ .

youngest leaves, while the older ones near the centre of the beet belong to the outer leaves. Since the leaf-bearing part of the axis is not elongated, the leaf-traces occurring on the same or approximately the same orthostichies must cross each other at some point which lies in a zone surrounding the apex, a short distance below the top of the beet (Fig. 10). The vascular anatomy in this zone is very confused. An additional complication is afforded by the fact that frequently, when the leaves are older, and have become pushed out to the periphery of the crown of the beet, a branch grows out from the original leaf-trace and forms an arc of vascular tissue outside the youngest trace on the same orthostichies (Fig. 1). Probably this accounts for certain irregularities in the vascular rings farther down from the apex.

In spite of these factors, however, the physiological individuality of the leaf-trace throughout the swollen organ is maintained, and this is reflected in the distribution of disintegrated tissue in the boron-starved plant. The youngest leaves first become blackened (Fig. 11), and hence the outermost ring of vascular tissue belonging to them first shows signs of disintegration. Although the traces pass very close to one another, they

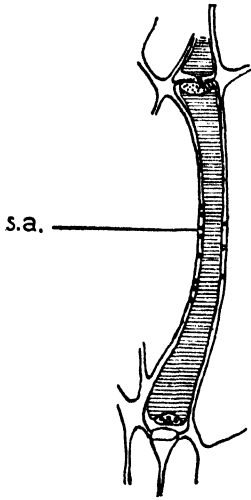


FIG. 9.

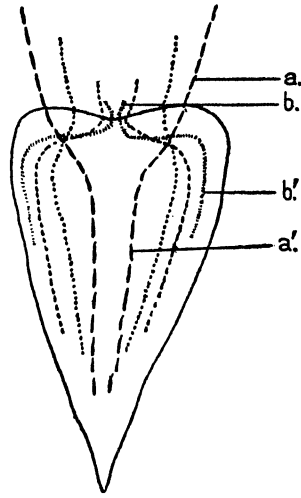


FIG. 10.

FIG. 9. A sieve-tube from a diseased plant, in longitudinal section, showing a mass of deeply staining substance filling the whole lumen. *s.a.* pits of sieve-areas on the sieve-walls.  $\times 990$ .

FIG. 10. Diagram to show the general course of the leaf-traces in the sugar-beet. Neither the primary vascular strand of the seedling nor the accessory strands branching off from the older leaf-traces are shown. *a.* Oldest leaf. *a'.* Oldest trace. *b.* Youngest leaf. *b'.* Youngest trace.

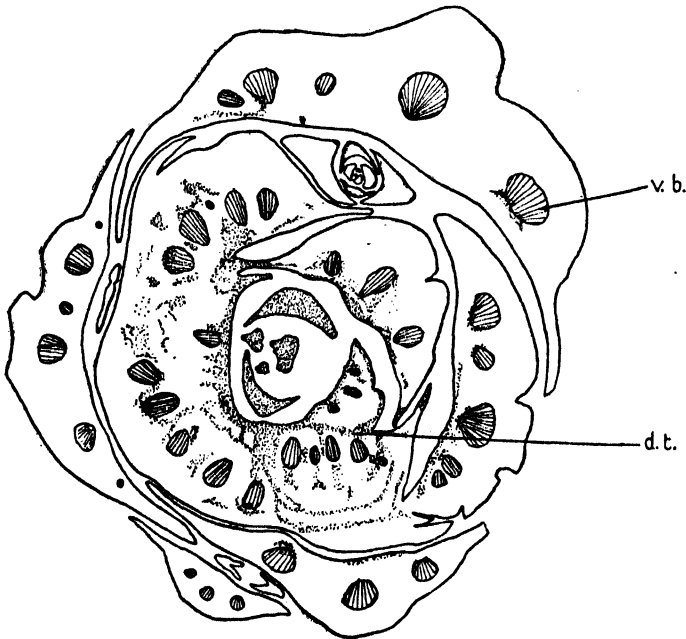


FIG. 11. Diagram of transverse section from just above the shoot apex, showing disintegration in the youngest leaves and the centrifugal spread of the disease in a plant that had been deprived of boron for three weeks. The diseased tissue is dotted. Vascular bundles are indicated in outline with line shading. *d.t.* diseased tissue. *v.b.* vascular bundle.  $\times 16$ .

do not 'contaminate' each other. This explains why 'heart-rot' is at first peripheral in the root-stock, and appears in the core only in the later stages (cf. Fig. 7).

The growth of axillary buds which follows after restoring boron to the nutrient solution is accompanied by the development of new cambia in the ground tissue of the hypocotyl and root. This is seen in set D (Fig. 2). There is no renewal of activity in or near those vascular zones which began to degenerate at the onset of degeneration in the leaves to which they belonged (Fig. 4). Again, the individuality of each leaf-trace of the axis is clear, for it is evident that the growth of the leaf-trace is dependent on the well-being of its own particular leaf, and no matter how much fresh growth is activated in the formation of new cambial zones, the single fact of the death of the leaf renders impossible any further development of its axial component. It is also brought to light that, in the axis, the repercussions of leaf activity include not only control of the growth of the axis, but also immediate transmission of pathological conditions, since the leaf-trace does not merely cease activity in the absence of boron, but is quickly converted into an isolated strand of diseased tissue. The undoubted connexion between the zones of secondary tissue and the leaves, together with the evidence here provided of the inability of the former to develop if the growth of the latter is checked, suggests that the axial part of the plant might be regarded as a number of fasciated leaf-traces. This can be demonstrated only in plants such as *Beta* where individual strips of cambium are associated with each leaf, and is impossible in most dicotyledons where there is only one cambium which is stimulated afresh into activity with the development of each leaf.

#### SUMMARY.

1. An experiment is described which confirms the results of earlier investigators, showing that boron is an essential element in the growth of sugar-beet. A concentration of 1 : 1,000,000  $H_3BO_3$  in the culture solution is enough for active growth and normal development.

2. It is shown that recovery in boron-starved plants involves the activation of axillary buds at the top of the beet, each of which develops its own system of secondary vascular rings independently of the others.

3. Attention is drawn to the fact that the secondary vascular zones in the beet are downward continuations of the vascular supply of the leaves, and that the influence of any factor adversely affecting the leaves is very quickly reflected in the corresponding vascular rings of the beet.

4. The apical meristem of the shoot, together with the youngest leaves and the newly developed cambia of the beet are tissues and organs which are most sensitive to boron-deficiency, and these are the first to degenerate. Cells of the vascular rings in process of differentiating, and

sporadic groups of parenchyma cells adjacent to conducting elements are also sensitive to the deficiency. Hypertrophy of the cambial cells, and also of the adjacent parenchyma cells, together with complete disintegration of the phloem, characterizes the later stages of heart-rot disease. It is suggested that plugging of the sieve-tubes is the first indication that the tissue is suffering from boron-deficiency.

5. Since the root-tip does not degenerate, but merely ceases to grow in the absence of boron from the nutrient solution, either the requirements of this meristem, or the conditions obtaining in it must be different from those of the shoot-apex.

In conclusion, the author wishes to thank Dr. W. E. Brenchley for her very helpful advice and criticism throughout the experiment.

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# Contributions to the Study of *Ceratostomella fimbriata*.

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With Plates XIII and XIV and fifteen Figures in the Text.

IN 1933 Andrus and Harter (2) published their observations on a pyrenomycetous fungus, *Ceratostomella fimbriata* (Ell. and Hals.) Elliot. In the following year Gwynne-Vaughan and Williamson (7) in a paper on *Ascophanus Aurora* had occasion to comment on some of their conclusions. Thereupon Dr. Andrus, in October 1934, most courteously forwarded to us a culture of the fungus and invited us to examine its cytology. His action has resulted in the present study.

The fungus occurs in nature as the cause of mouldy rot on Para rubber trees and of black rot on sweet potatoes; it grows readily in culture at temperatures ranging from 19° to 30° C. and gives rise to chlamydospores, endoconidia, and ascospores in perithecia. The ascospores and endoconidia, in turn, germinate freely and produce ascocarps in single spore cultures of either origin. Fruiting takes place indifferently in light or darkness.

Several culture media were employed, by far the most successful being potato agar. Potatoes were cut up without removing the skins, they were boiled, the liquor strained off, and 1 per cent. of pure glucose added. The purity of this substance is important, as the commercial product contains harmful ingredients.

When fully satisfactory conditions of growth had been established material was fixed every hour. Here, however, a difficulty arose, opening the incubator to remove the first cultures to be fixed, adversely affected the growth of the remainder. In order to obtain thoroughly healthy and rapidly growing material it was therefore necessary to keep each culture intended for fixation in a separate incubator. A certain amount of material was grown in the open at about 26° C. but such material is difficult to protect from chance variations in its environment, and to these the fungus is very sensitive. Much air is entangled among the hyphae and, when non-alcoholic fixing fluids are used, has to be removed by the energetic use of an exhaust

pump. Since this can be done more effectively when the material is in a bottle than in a desiccator, the agar was cut across before inoculation so that the pieces bearing the fungus could be quickly transferred to a fixing bottle as soon as the incubator was opened. It cannot be too strongly emphasized that rapid fixation of undisturbed material is essential if active and healthy stages are to be obtained.

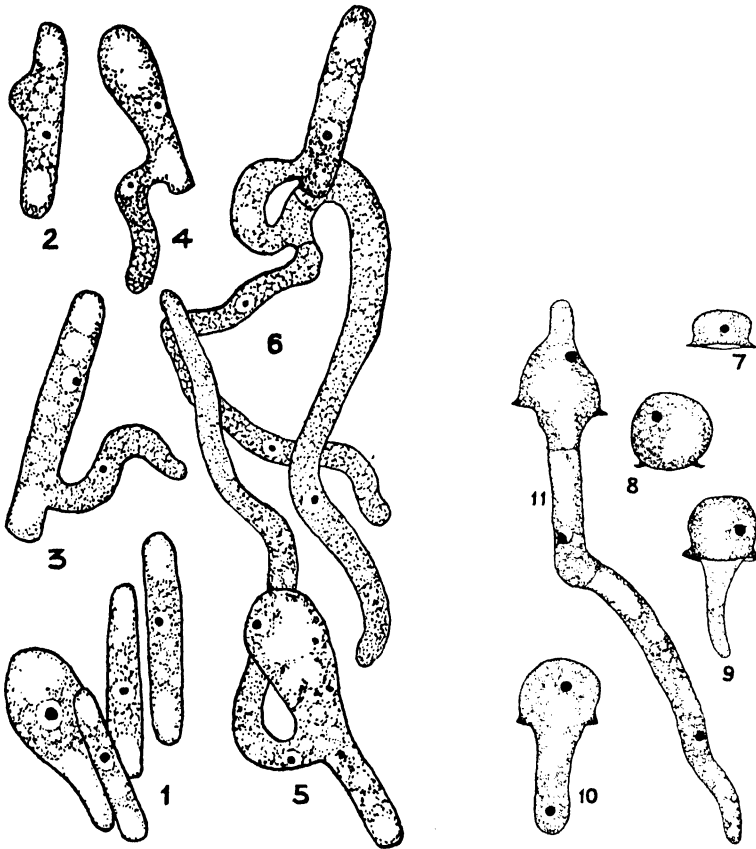
The greater part of the material was fixed for twenty-four hours in Fleming's strong fluid diluted with an equal quantity of water. The fixative known as 2 BD (9) was also found successful and, owing to the transparency of the cytoplasm, was of value for certain aspects of microphotography. Carnoy's and Bouin's fluids gave relatively poor and unreliable results. The effect of cooling or warming the fixing fluid was explored, temperatures from 0° to 40° C. being employed, but no advantage was found to have been gained, indeed fixation in the warmer fluids was definitely inferior.

Sections, for the most part, were cut  $7\mu$  in thickness, bleached with chlorine and stained in iron haematoxylin followed by erythrosin in clove oil. The best results were obtained when the slides, having been freed from alcohol and mordanted with iron alum, were well rinsed in distilled water and placed for twenty-four hours in 0.05 per cent. haematoxylin. They were then thoroughly washed with distilled water, washed out under the microscope in saturated aqueous solution of iron alum to which a few drops of glacial acetic acid had been added, and left for ten or fifteen minutes in running tap water before being taken through alcohol to the counterstain. Finally the clove oil was removed with cedar oil and the sections mounted in Sira medium. The unusual dilution of the haematoxylin, 10 per cent. of that commonly employed, and the strong solution of iron alum for washing out give a brilliant and very delicate stain. Owing to the slight alkalinity of tap water, it is undesirable that this should be used till staining is complete.

All forms of spore germinate without a resting stage. The nucleus divides (Pl. XIII, Fig. 3) showing three chromosomes, the haploid number. The endoconidia send out a single, lateral germ tube (Text-figs. 2-5) which soon branches (Text-fig. 6), the germ tube from the chlamydospores is terminal. The ascospores are of the form usually described as bowler-hat shaped (Text-fig. 7), they enlarge considerably (Text-fig. 8) and put out the first germ tube (Text-figs. 9, 10) from within the circle of the 'brim'. Nuclear division takes place, one of the nuclei remains in the spore, the other passes into the germ tube. Soon a second germ tube (Text-fig. 11) emerges from the 'crown' of the hat.

Since the germ tubes are effectively uninucleate, septation associated with mitosis results in a series of uninucleate segments. The mycelium derived from the chlamydospores, endoconidia, and ascospores thus consists of cells with a single nucleus, and though occasional fusions may take place between hyphae, a multinucleate condition is not thereby established.

During active growth vacuoles are to be found in the neighbourhood of the transverse septa (Text-fig. 11) indicating that, as in the ascogenous hyphae (5, 6, 7) septa are formed across the parental nuclear area between the daughter nuclei. The development of the septum is of the type usual in



TEXT-FIGS. 1-6.

TEXT-FIGS. 7-11.

TEXT-FIGS. 1-6. Fig. 1. Group of endoconidia from same conidiophore, the first to be extruded is somewhat enlarged at the distal end, the others have parallel sides. Figs. 2-6. Stages in the germination of endoconidia.  $\times 1,600$ .

TEXT-FIGS. 7-11. Fig. 7. Ripe ascospore. Fig. 8. Ascospore enlarged in preparation for germination. Figs. 9-11. Germinating ascospores. Figs.  $\times 1,600$ .

Thallophyta, beginning with an annular intrusion in contact with the lateral walls and gradually spreading across the cell.

Some eight hours after the germination of a spore has begun, endoconidiophores appear (Pl. XIII, Fig. 1) and uninucleate endoconidia (Pl. XIII, Fig. 2) are extruded. The first of each series is somewhat enlarged at its distal end (Text-fig. 1), its successors have parallel sides.



Chlamydospores also soon begin to appear. They have been fully described by Andrus and Harter (2) and need no further comment.

At 25° C. the primordia of ascocarps may be recognized in cultures nineteen or twenty hours old. A short, fertile hypha, rich in contents, becomes curved (Pl. XIII, Fig. 4) and septate (Text-fig. 12) and from its lower cells (Text-figs. 13, 14) branches are developed (Pl. XIII, Fig. 5) and ramify to form a sheath (Pl. XIII, Fig. 6). In Pl. XIII, Fig. 5 these sheath branches are still thin walled, in the stage shown in Pl. XIII, Fig. 6, differentiation has begun, an outer layer of thick-walled cells with scanty contents can be distinguished and a few thin-walled inner cells. In Text-fig. 15 the inner layer has developed somewhat further, and in Pl. XIV, Fig. 19, it is already well established.

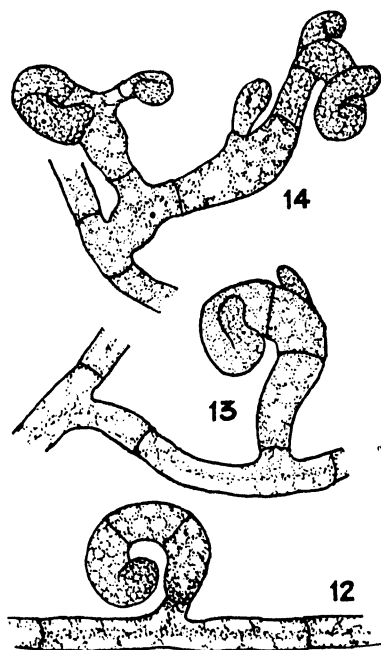
The cells of the fertile branch are at first uninucleate, like those of the vegetative hyphae, but, sooner or later, one of them is seen to contain two nuclei. This condition may appear very early (Pl. XIII, Fig. 4) before branches are formed and when it is evident that cell fusion has not occurred, so that the nuclei are clearly the products of mitosis, or it may be delayed till sheath hyphae are present. The enlargement and curvature of the hypha forming the fertile branch makes it possible for a spindle to lie obliquely; nuclear division, under such circumstances, can be completed without relation to the containing walls. This may well be the cause of failure of septation. Once a cell is binucleate any further mitosis, if accompanied by septation, will result in one daughter nucleus of each division being cut off in an end cell while the other two remain in the middle region. When septation again fails, as it may if the cell enlarges, the binucleate cell will become multinucleate. Associated binucleate and uninucleate cells are to be seen in Pl. XIII, Fig. 6, and in Text-fig. 15, in Pl. XIV, Fig. 19, the multinucleate condition is attained.

At no stage during the development of the fertile branch was there any evidence of the presence of an antheridium or trichogyne, nor were any cell or nuclear fusions observed.

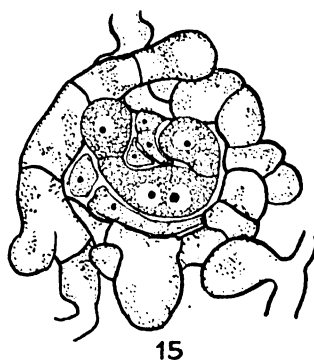
The multinucleate cell contains a variable number of nuclei, and may branch or divide to form multinucleate segments (Pl. XIV, Fig. 20). Before long, however, more delicate branches are differentiated, and at the stage shown in Pl. XIV, Fig. 21, the ascogenous hyphae may be recognized as groups of binucleate and multinucleate cells. They are thus of the same type as the ascogenous hyphae described (5, 6, 7) in other species. With their development the multinucleate cells are used up and disappear.

*The sheath.* By the time that the multinucleate cells have given place to ascogenous hyphae the sheath is seen to consist of three zones. The thick-walled cells already recognizable in Pl. XIII, Fig. 6, now form two to four external layers, within which are two or more layers of thin-walled cells, constituting the inner sheath, while the perithecium is lined by one

or two layers of cushion cells (Pl. XIV, Figs. 22, 26, 27). These, as development proceeds, become increasingly distended by the contents of their large vacuoles, and serve as a sort of water-bed against which the proximal parts of the ascogenous hyphae lie, while their distal ends extend into the



TEXT-FIGS. 12-14.



TEXT-FIG. 15.

TEXT-FIGS. 12-14. Fig. 12. Young fertile branch. Fig. 13. Fertile branch with first hypha of sheath. Fig. 14. Two fertile branches arising from a dichotomy, both with lateral branches. All from uncut material.  $\times 1,600$ .

TEXT-FIG. 15. Section through very young perithecium. Three cells of the fertile branch, one binucleate, are visible in the middle, near them lie a few cells of the inner sheath, the outer sheath, already somewhat thick walled, surrounds the whole.  $\times 1,600$ .

cavity of the perithecium. During the growth of the asci the cushion cells lose their turgidity and become flattened (Pl. XIV, Figs. 27, 28) against the surrounding layers of the sheath. As a consequence the perithecial cavity, already enlarged by the growth of its walls, is still further increased in size. The ascogenous hyphae and young asci appear to be still connected with the sides of the cavity, but, since they grow inwards from every direction, and can only be examined in section, it is difficult to state this with precision.

*The neck.* Already in Pl. XIV, Fig. 19, the future position of the neck is indicated by a slight upward tendency of the cells of the inner sheath. This becomes more marked, the contents of the cells increase in density,

and, by the time that the multinucleate fertile cell has given rise to multinucleate segments the perithecial cavity can be identified, (Pl. XIV, Fig. 20) and is surmounted by a well-marked meristematic region. While the enlargement of the cushion cells is in progress the meristematic hyphae of the neck push outwards (Pl. XIV, Fig. 21), and, converging from every side, form with their distal tips a hollow tube. At this stage (Pl. XIV, Fig. 22) the cells of the outer sheath are being pushed apart, and the neck emerges (Pl. XIV, Fig. 23) as a cone of thin-walled hyphae.

Up to this point the hyphae which constitute the neck have developed in the same way, and served the same purpose as the first cone of paraphyses in a discomycetous ascocarp. Their converging apices have pushed aside the outer sheath and blazed the trail for the escape of the ascospores. From now onwards their behaviour is different. The discomycetous paraphyses elongate no further, but, as they spread outwards and increase in number, progressively enlarge the aperture of the fruit. In *C. fimbriata*, on the other hand, the meristematic hyphae increase in length, and the walls of their three outer layers become thickened, forming a firm, tubular extension. The expanded base is lined by several layers of delicate cells (Pl. XIV, Fig. 33), which are extended upwards as a single layer (Pl. XIV, Figs. 32, 34) throughout the length of the tube. Meanwhile, within the perithecium, the converging hyphae have overlapped less closely (Pl. XIV, Fig. 30), a passage has appeared between them (Pl. XIV, Fig. 31), and they are recognizable as periphyses. The hyphae of the neck continue their apical growth till a total length of about 1 millimetre above the substratum is reached, and the neck itself is from 0.5 to 0.7 millimetres long; they then spread apart, giving the fimbriated appearance from which the name of the species is derived. The development of the neck, at 25° C., occupies about three days, elongation occurring in the last seventeen to twenty-three hours.

*The asci.* The first asci may be recognized at about the time of the elongation of the neck, the stage shown in Pl. XIV, Fig. 24. They arise from the binucleate cells of the ascogenous hyphae, among which the usual croziers (Pl. XIII, Fig. 7) are to be seen. The definitive nucleus of the ascus (Pl. XIII, Fig. 8, shows a large chromatin body and a delicate reticulum, which is often difficult to recognize. Uninucleate asci are exceedingly common, some perithecia showing little else (Pl. XIV, Fig. 27), so that, as usual, this condition may be inferred to last for some time before meiosis begins. Three gemini then become visible (Pl. XIII, Fig. 9), they pass on to the spindle (Pl. XIII, Fig. 10), and three daughter chromosomes (Pl. XIII, Fig. 11) travel to each pole (Pl. XIII, Fig. 12). Asci containing two or four nuclei are rare, indicating that the second (Pl. XIII, Figs. 13, 14) and third (Pl. XIII, Figs. 15, 16) divisions quickly follow the first. In both the chromosomes are minute, and we did not find it possible to count them. Since, however, three is the haploid number as well as the number of

gemini, it is evident that, in this fungus, as in *Neurospora tetrasperma* (3), no further reduction follows meiosis.

Both before and during the meiotic phase and the following vegetative division the cytoplasm of the ascus is markedly vacuolate, sometimes showing a foamy structure (Pl. XIII, Figs. 10, 11; Pl. XIV, Fig. 29), sometimes a single, large, very well-defined vacuole (Pl. XIII, Fig. 8; Pl. XIV, Fig. 35), surrounded by a denser zone. If the ascus is so cut that the observer looks through the vacuole at the nuclei (Pl. XIII, Fig. 16), a peculiar effect is produced, the nuclei seeming to be surrounded by a secondary vesicle. That such an appearance is not constant is clear from Pl. XIII, Figs. 12-15, and is also indicated in Pl. XIV, Fig. 29.

In the ascus the developing spores (Pl. XIII, Figs. 17, 18) lie in four rows of two spores each, two of the rows being nearer one end of the ascus and two somewhat nearer the other. Before the spores are shed the flattened extension, which forms the 'brim' of the hat-shaped spore, is readily recognized (Pl. XIII, Fig. 18). The ascus wall soon disappears, but the spores lie for some time in pairs, brim to brim. They are uninucleate (Text-fig. 7), usually with a well-marked vacuole. They pass up the neck (Pl. XIV, Figs. 32-4) singly or in pairs, and are held for a while in a drop of mucilage outside the ostiole.

#### DISCUSSION.

In his account of *C. fimbriata* in 1925 Elliott (4) interpreted the perithecial rudiment as a straight oogonial branch around which an antheridium became twisted. Mittmann (10), in 1932, recorded a coil of uninucleate cells often ending in a long cell which contained more than one nucleus. Andrus and Harter (2), in the following year, also observed a coiled branch, but they described as antheridial the first lateral outgrowth from this fertile hypha, though they held that it did not function. We can confirm their description and that of Mittmann of the fertile branch, and we are agreed that fertilization does not take place. But we are satisfied that no recognizable antheridium is present, and that the first lateral outgrowth from the fertile hypha takes part, like those produced later, in the formation of the sheath. We are in accord with Mittmann, and also with Andrus and Harter, in finding that a median cell of the fertile coil becomes binucleate and later multinucleate, that it may branch or divide to form other multinucleate elements, and that from these the asci are ultimately derived.

Andrus and Harter figure the cytoplasm of the multinucleate fertile cell as detached from the surrounding walls, they are of opinion that its wall is dissolved, and that it gives rise to naked, binucleate protoplasts lying detached in the cavity of the perithecium and embedded in a nutritive matrix. Ultimately in some of these cells they find that two nuclei fuse, and such cells enlarge to become the uninucleate, but still naked asci.

They found no development of ascus hooks. They describe the first division in the ascus as characterized by the formation of an ascus vesicle, the limiting layer of which they regard as the enlarged membrane of the definitive nucleus. They describe the three divisions in the ascus and the subsequent production of spores as taking place within this membrane, which finally expands to coincide with the external limits of the protoplast, and to become, in fact, the wall of the ascus.

In so far as we have observed the separation of the cytoplasm of the fertile cell from the corresponding wall, we are satisfied that it is due to the contraction which takes place all too readily during the fixation of delicate structures enclosed in a thick, perithecial sheath. The cell-wall, however, is not lost, and, in well fixed preparations, the cytoplasm remains in close contact with it. The ascogenous hyphae and the asci at all stages of development we found to possess the usual delicate wall, staining convincingly with erythrosin in clove oil. The curious vesicle figured by Mittmann and by Andrus and Harter, and regarded by the latter authors as the expanded nuclear membrane of the definitive nucleus, we are inclined to identify with the large vacuole which, as we have already shown, may produce the illusion of enclosing the nuclei of the ascus.

We have seen no evidence of digestion within the perithecium as described by Andrus and Harter, but we agree with them that considerable enlargement of the perithecial cavity takes place, and we attribute this, apart from the growth in progress, to the collapse of the cushion cells. Cushion cells are recognizable in Andrus and Harter's Pl. I B (2).

Varitchak (12) in *C. piceae*, Sartoris (11) in *C. adiposum*, and Mittmann in *C. coerulea* and *C. quercus* have shown fertile branches and ascogenous hyphae similar to those here described. In *C. multiannulata* Andrus (1) has lately figured perithecial primordia of the same type. He observed in the fertile branch a cell with two nuclei accompanied by a terminal and a basal uninucleate cell. A consideration of the origin of the binucleate condition he has reserved for a later paper. The binucleate cell elongates, forms a coil, and becomes multinucleate. Simultaneous division takes place in two or more of the nuclei. Later, when surrounded by a sheath of three or four layers, the multinucleate cell divides and forms a quantity of independent cells containing two or four nuclei. From these the asci are derived, sometimes being preceded by the appearance of a crozier, sometimes linked together. The usual three successive divisions were observed in the ascus, and, in this species, the membrane of the definitive nucleus was found to disappear after the first mitosis in the usual way. From an early stage of development the fertile cell and its products, including the asci themselves, are described as without walls, though a delicate surface membrane, presumed to be protoplasmic in character, was sometimes recognized. Counterstains, which are usually taken up by the wall, do not appear to

have been employed. During development the lower part of the perithecium is occupied by cells defined as pseudoparenchymatous, which appear to correspond to our cushion cells, but to be limited to the basal region. There is no sign of the presence of cushion cells in Varitchak's figures of *C. piceae*.

In the development of the neck Sartoris (11), for *C. adiposum*, describes a meristematic area at the apex of the perithecium composed of hyphae arising from opposite points on the inner wall. They grow at first towards one another and, when only a small passage remains, turn upwards and run parallel. At the same time the thick-walled hyphae from the outer sheath of the perithecium envelop and extend beyond them, the whole forming a compact, fasciculated, rigid structure. Later the central, thin-walled cells are dissolved, leaving a canal delimited by layers of thick-walled filaments. At maturity some of these extend beyond the ostiole as a fringe. The mature, crescent-shaped spores and the fatty substance in which they are embedded fill the cavity of the perithecium and hydrostatic pressure develops. Some of the spores are forced up the neck, gathering in a translucent droplet. The remainder are not distributed till the perithecium disintegrates.

In *C. piceae* Varitchak (12) describes the asci and later the spores as lying free in the cavity of the perithecium, so that the latter is filled with mucilaginous material which exudes with the spores from the neck. His figures of the neck, like those of Sartoris, indicate that the outer sheath furnishes its external hyphae. This is also suggested by the figures of Andrus (1) for *C. multiannulata*. Ingold (8) has examined the discharge of spores in *C. ampullasca* and finds that the perithecial cavity is crowded with detached asci, held upright by mutual pressure. Entire asci in this species pass up the neck and shoot their spores from the ostiole. Ingold does not deal with the development of the neck.

Andrus and Harter (2) are not concerned with the growth of the neck in *C. fimbriata*, but their admirable figure of an entire perithecium, seen from without, suggests the emergence of the neck which we have described in this species.

It is evident that the method of spore discharge differs in different species of the genus, showing what is probably a degeneration series from *C. ampullasca*, through such forms as *C. fimbriata* to *C. adiposum*. There is variation also in the development of cushion cells and apparently in the origin and development of the neck.

It is a rather surprising detail that, in *C. fimbriata*, neither Mittmann nor Andrus and Harter refer to the very characteristic shape of the ascospores.

## SUMMARY.

1. *Ceratostomella fimbriata* (Ell. and Hals.) Elliott is self-compatible, bearing endoconidia, chlamydospores, and ascospores in perithecia in single spore culture. The development and germination of the spores are described. The mycelium consists of uninucleate cells.

2. The perithecium initial is a curved hypha, one of the cells of which becomes binucleate by mitosis without wall formation, and later multinucleate. No antheridium was seen, and there is no evidence of fertilization.

3. The multinucleate cell gives rise to several smaller multinucleate cells. These branch to form ascogenous hyphae made up of binucleate and uninucleate cells. The usual ascus hooks are formed.

4. The multinucleate cells, the cells of the ascogenous hyphae and the asci possess the usual delicate cell-walls. No naked cells were recognized.

5. Meiosis takes place in the first divisions in the ascus showing three gemini. The haploid number of chromosomes as seen in germinating spores is three. This confirms the evidence for the absence of fertilization. Brachymeiosis is also omitted.

6. The cytoplasm of the ascus is vacuolate. A large vacuole sometimes produces the illusion that the ascus nuclei are enclosed within it.

7. The perithecial sheath has an outer zone of thick-walled cells and an inner zone of thin-walled hyphae. The cavity is lined by cushion cells which become turgid during the development of the ascogenous hyphae and later collapse.

8. The neck originates from a meristematic region of the inner sheath. It pushes through the outer sheath and elongates. Its external cells form a rigid tube lined by a layer of delicate hyphae.

9. We are able to confirm many of the observations of Andrus and Harter with regard to the accessory spores, the initiation of the perithecium, and the structure of its sheath.

The fungal material for this work was derived from a culture received from Dr. C. F. Andrus of the Bureau of Plant Industry, Washington, to whom our thanks are tendered.

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## EXPLANATION OF PLATES XIII AND XIV.

Illustrating Professor Gwynne-Vaughan and Mrs. Q. E. Broadhead's paper on Contributions to the Study of *Ceratostomella fimbriata*.

### PLATE XIII.

(All figures are drawn from microtome sections.)

- Fig. 1. Endoconidiophore after emission of first conidium. × 1,600.
- Fig. 2. Upper part of an endoconidiophore. One of the later formed conidia is in process of liberation. × 1,600.
- Fig. 3. Mitosis in a germinating chlamydospore showing three chromosomes moving towards each pole of the spindle. × 2,600.
- Fig. 4. Very young fertile hypha with two nuclei in recurved, terminal cell. × 1,600.
- Fig. 5. Section through an older fertile hypha to show first branches of sheath. × 1,600.
- Fig. 6. Lateral view of fertile hypha surrounded by cells of sheath. The penultimate cell contains two nuclei. × 1,600.
- Fig. 7. An ascogenous hypha with four nuclei. × 2,600.
- Fig. 8. Uninucleate ascus showing large vacuole below which lies the nucleus with nucleolus and delicate reticulum. The same ascus is reproduced in Fig. 35 on Pl. XIV. × 2,600.
- Fig. 9. Prophase of meiosis in ascus, showing three gemini. × 2,600.
- Fig. 10. Early metaphase of meiosis with three gemini passing on to spindle. × 2,600.
- Fig. 11. Anaphase of first meiotic division, three chromosomes are clearly visible travelling towards upper pole of spindle. × 2,600.
- Fig. 12. Telophase of first meiotic division, the nuclear membrane has disappeared. × 2,600.
- Fig. 13. Anaphase of second division in ascus. × 2,600.
- Fig. 14. Telophase of second division in ascus. × 2,600.
- Fig. 15. Telophase of third division in ascus. × 2,600. The same ascus appears in Figs. 28 and 29, Pl. XIV.
- Fig. 16. Telophase of third division in ascus, seen through a vacuole such as that shown in Fig. 8. × 2,600.
- Fig. 17. Ascus with developing spores. Four uninucleate spores are visible and behind them parts of three of the four others. × 2,600.
- Fig. 18. Longitudinal view of ascus containing eight spores. The characteristic hat shape is already recognizable, the 'brims' lying between the pairs of spores. × 2,600.

### PLATE XIV.

(All figures are reproduced from untouched photographs.)

- Fig. 19. Very young perithecium with multinucleate fertile cell and first indication of neck. The outer and inner layers of the sheath are recognizable. × 500.



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Fig. 20. Young perithecium, with several smaller, multinucleate cells, showing meristematic hyphae of neck and beginning of perithecial cavity.  $\times 500$ .

Fig. 21. Perithecium with ascogenous hyphae. The elongation of the neck and the enlargement of the cushion cells are beginning.  $\times 500$ .

Fig. 22. Perithecium just before emergence of neck. The tips of the neck hyphae are directed slightly towards the observer, so that the apex is seen in surface view. The cushion cells are further enlarged.  $\times 500$ .

Fig. 23. Perithecium just after emergence of neck. The cushion cells have reached their full size.  $\times 500$ .

Fig. 24. Perithecium with young asci. The neck is further developed, it is somewhat curved in the plane of section, so that the middle region is cut while the tip is entire.  $\times 350$ .

Fig. 25. Apex of elongating neck to show circle of parallel hyphae.  $\times 500$ .

Fig. 26. Perithecium slightly older than that in Fig. 6. The cushion cells are still turgid and the asci uninucleate.  $\times 500$ .

Fig. 27. Rather older perithecium still with mainly uninucleate asci. The cushion cells are beginning to lose their turgidity. The sheath is unusually thick.  $\times 500$ .

Fig. 28. Older perithecium. The cushion cells have collapsed, the periphyses at the base of the neck are beginning to separate. Uninucleate asci can still be seen, but already free spores are present as well as asci containing spores and an ascus at *a* with nuclei in mitosis.  $\times 500$ .

Fig. 29. Enlargement of a part of Fig. 28 show the ascus at *a* with nuclei in mitosis. The same ascus with greater depth of focus is represented in Pl. XIII, Fig. 15.  $\times 2,600$ .

Fig. 30. Median longitudinal section through base of neck showing newly separated periphyses.  $\times 500$ .

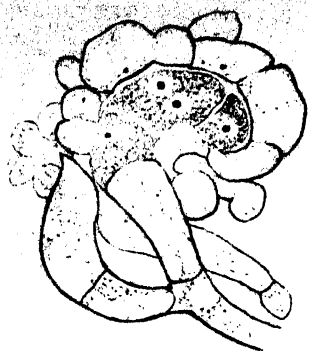
Fig. 31. Median longitudinal section through base of older neck; the central passage is well established.  $\times 500$ .

Fig. 32. Longitudinal section through part of neck, showing outer, thick-walled cells, delicate lining and median canal with spores.  $\times 500$ .

Fig. 33. Transverse section through base of neck; deeply stained spores are present in the canal.  $\times 500$ .

Fig. 34. Transverse section through upper part of neck, showing thick-walled outer layers symmetrically arranged and delicate lining. A spore occupies the canal.  $\times 500$ .

Fig. 35. The ascus shown in Pl. XIII, Fig. 8, but in rather different focus. A large vacuole is visible above, and a nucleus, with nucleolus, below. A shadow due to an ascus in different focus is thrown across part of the vacuole.  $\times 2,600$ .



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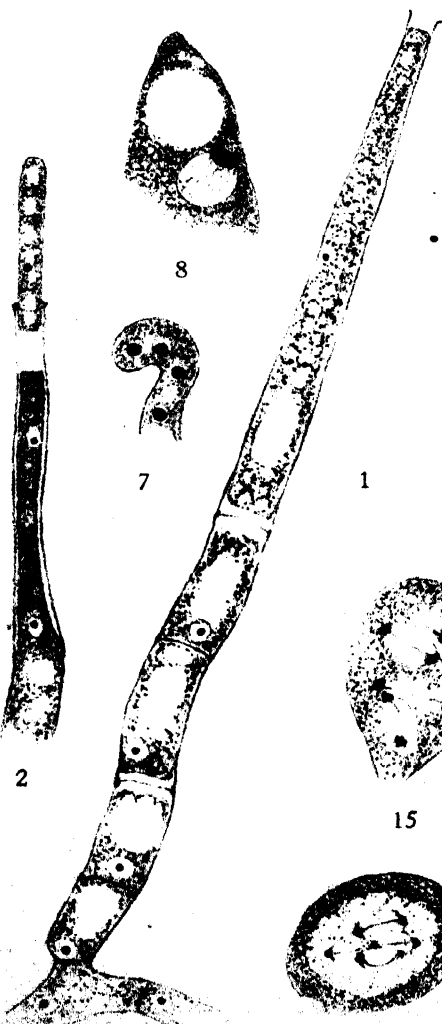
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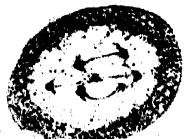
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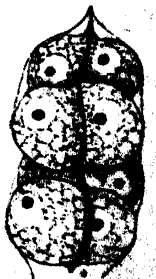
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# Observations on the Floral Fibres of Certain Gentianaceae.

BY

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With Plates XV and XVI and three Figures in the Text.

## INTRODUCTION.

WHILE botanizing in the vicinity of Juan Diaz, Republic of Panama, several years ago, I encountered a herbaceous member of Gentianaceae, *Coutoubea spicata* Aubl., flowers of which were preserved in formalin-acetic-alcohol for subsequent anatomical investigation in relation to a survey of the floral morphology of the entire family which is still uncompleted. Upon sectioning after the usual xylol-paraffin series in embedding it was found impossible to obtain even moderately thin transverse slices because of the presence of peculiarly lignified bundles which occur in the pedicel, calyx, corolla, and staminal filaments. Shortly later preserved material of the flowers of *Lisianthus brevidentatus* (Hemsl.) Perk. was sent me from British Honduras by Mr. William A. Schipp, together with additional material of *C. spicata*. The *Lisianthus* flowers proved to be virtually as difficult to section as those of *Coutoubea*. Interest aroused by such unforeseen difficulties prompted thereupon a detailed study of the composition and ontogeny of the peculiar floral bundles of the two species, which are apparently representative of similar conditions in all species of the respective genera.

*C. spicata* is a herb apparently rather common in Central America and north-eastern South America rarely attaining a height of over 1 metre. The small flowers, including a salver-form corolla somewhat less than 1 cm. in length, are aggregated quaternately into a fairly elongate, bracteate, spicate raceme. Immediately subtending the nearly sessile flowers are two to four closely decussate bracts in the axils of which abortive, lateral buds are occasionally found in microscopical preparations, evidently indicating the derivation of the inflorescence from a thyrses (4). *C. ramosa* Aubl., the remaining species of the genus, has a general habit similar to that of *C. spicata* and the flowers are likewise characterized by similar bundles, although the inflorescence is more diffuse and, with the exception of the decussate anthotaxy, is nearer anatomically to the generalized raceme. It

[Annals of Botany, Vol. L. No. CC. October, 1936.]

is a species apparently limited in distribution to north-eastern South America.

*L. brevidentatus* is likewise herbaceous, but the inflorescence is a lax dichasium of rather regular composition. In both *Lisianthus* and *Coutoubea* the corolla is more or less persistent, twisting about the small capsules, when the peculiar bundles of the corolla members are particularly noticeable in *exsiccatae* (Pl. XV, Fig. 1).

Within relatively recent years the inclusive genus *Lisianthus* has been subjected to repeated segregation, particularly by the late Dr. E. Gilg. It is of taxonomic interest therefore to note that only the typical element of the genus has been found to contain the extraordinary floral bundles observed in *L. brevidentatus*. Amongst a wide assortment of herbarium specimens representative of the genera *Chelonanthus*, *Purdieanthus*, *Macrocarpaea*, *Calolisianthus*, *Lagenanthus*, *Adenolisianthus*, all of Gilg, *Symbolanthus* G. Don., *Pagaea* Griseb., and *Bisgoeppertia* O. Ktze., similar conditions have not as yet been found. Other genera of *Gentianaceae* demonstrating similar floral bundles with greater or less clarity upon herbarium specimens include *Canscora* Lam., *Centaurium* Gilib., and *Zygostigma* Griseb.

#### MATERIALS AND METHODS.

For an anatomical study of the flowers material of *L. brevidentatus* and *C. spicata* was collected by Mr. Schipp in British Honduras. The *Coutoubea* species was also collected in preservative by Dr. J. A. Steyermark and by myself in Panama. In each case a wide assortment in floral development was preserved in the customary solution of formalin, acetic acid, and alcohol.

Several different embedding schedules, including celloidin, were followed in an attempt to obtain smooth microtome sections of 10  $\mu$  or less in thickness. In general, however, a preliminary treatment of 25 per cent. hydrofluoric acid in alcohol, followed by the usual xylol-paraffin series, proved more convenient and as advantageous in other respects. The proprietary fluid 'diaphanol' was somewhat less beneficial than the hydrofluoric acid. The butyl alcohol-paraffin method was also tried, but penetration appeared to be less perfect as a result. In any event soaking in water for about one day rendered the material embedded in paraffin blocks easier to section. The combination of aqueous crystal violet and erythrosin in clove oil was found to be satisfactory in staining.

In addition to microtome sections, maceration of the floral bundles was obtained for the study of the individual vascular elements. For that purpose the flowers were first dissected into convenient pieces and heated for about fifteen minutes in a vial containing Jeffrey's macerating fluid of chromic and nitric acids. Under a dissecting microscope the parenchyma

was then cleaned from the vascular bundles under tap water, and subsequently transferred to a 10 per cent. aqueous solution of caustic potash and heated as previously for about ten minutes. The elements were then teased apart in tap water and mounted for microscopic study. This vigorous treatment was quite necessary to dissociate the vascular elements and did not appear to damage them in the least.

## OBSERVATIONS.

*Coutoubea spicata* Aubl.

In a transverse section of the flower of *C. spicata* the early difficulty in sectioning referred to in a previous paragraph is found to be caused by sixteen to eighteen major bundles supplying the normally four corolline members and stamens together with the numerous similar bundles within the series of calyx lobes (Pl. XV, Fig. 2). These bundles are given off by a heavily vascularized pedicellar stele. The individual bundles as well as the pedicellar stele are found to be composed predominantly of numerous very compact, heavily lignified elements with little evidence of the surrounding phloem normally found in the bicollateral vascular system of most Gentianaceae (Pl. XV, Fig. 3).

At an early stage in this investigation the ontogeny of the vascular bundles of the flowers was traced through a wide assortment in floral development, details of which are recorded by drawings in Pl. XVI. In very young buds, somewhat less than 2 mm. in length, the vascular tissue is found to be undeveloped and the course of the future conductive tissues manifest by closely compacted provascular cells (Pl. XVI, Fig. 7). Such a condition is found in both calyx and corolla, as well as in the succeeding stages in development which follow.

Very soon thereafter a few strands of protoxylem are differentiated towards the centre of the provascular district and, dorsally, numerous small, closely compacted cells evidently referable to protophloem parenchyma (Pl. XVI, Fig. 8). Separating the protoxylem and the protophloem is a conspicuous provascular zone which soon commences differentiation to

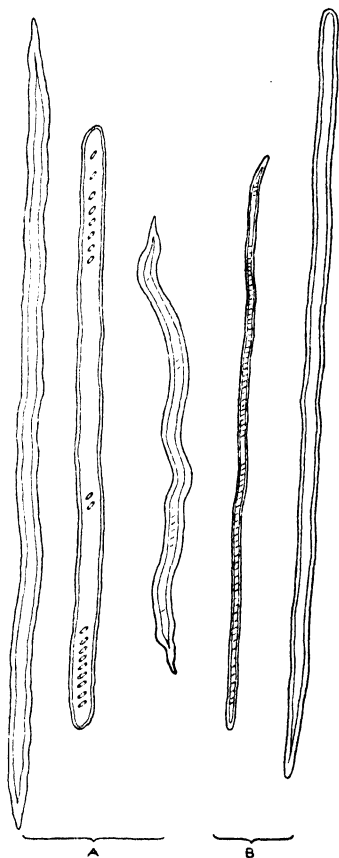


FIG. 1. A, metaxylary fibres macerated from the calyx bundles of *C. spicata* Aubl.; B, metaxylary fibres macerated from the corolla of the same species.

metaxylem in an irregularly centrifugal order. As differentiation of the metaxylem becomes complete the protophloem becomes crushed and ultimately completely absorbed (Pl. XVI, Fig. 11). This sequence of events is followed with approximate coincidence in the bundles of pedicel, calyx,

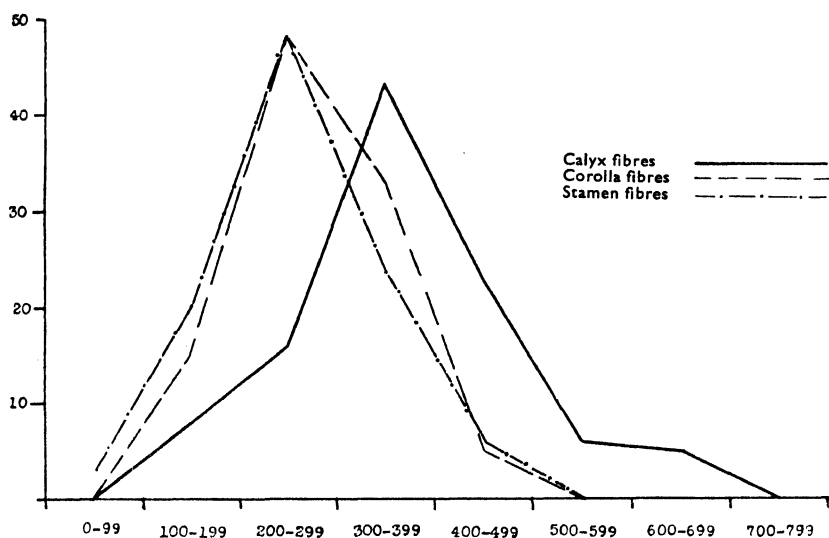


FIG. 2. Dimensional relations of the metaxylary floral fibres of *C. spicata* Aubl. One hundred fibres were measured for each curve. Vertical numerals refer to the number of fibres for each measurement; horizontal numerals to the length of fibres in micra.

corolla, staminal filaments, but only occasionally towards the base of the subtending bracts; and is noticeably absent in the gynaecium where normal bundles of protoxylem and protophloem alone are found. A peculiarity of the floral bundles that is at first overshadowed by the exaggerated development of the xylem is the fact that internal phloem is lacking, although present in the receptacle and the vegetative organs.

Longitudinal sections and macerations of the bundles of *C. spicata* reveal the fact that the lignified elements of the metaxylem of calyx, corolla, and staminal filaments, as well as of the receptacle and pedicel, are fibrous (Text-fig. 1). The sculpturing varies from rather small, oblique bordered pits with true perforations to oblique, evidently quite non-functional slits. Occasional elements are found which are completely destitute of any pitting.

Rather interesting dimensional relations of the floral fibres of *C. spicata* are summarized graphically in Text-fig. 2. The fibrous elements of the calyx range from 110-693  $\mu$  in length and from 7-20  $\mu$  in greatest width; those of the corolla from 131-468  $\mu$  in length and 8-23  $\mu$  in width; and those of the staminal filaments from 72-481  $\mu$  in length and 6-18  $\mu$  in width. The calycine fibres therefore exhibit not only greater length, but greater variation in length, than do those of the corolla and staminal filaments

which are practically equivalent. It is interesting, furthermore, to note in this connexion that the length of the floral fibres of *Coutoubea*, evidently metaxylem, accords in general with the dimensional statistics given by Bailey and Tupper (2) for the secondary tracheides and fibres of related families of woody Angiosperms.

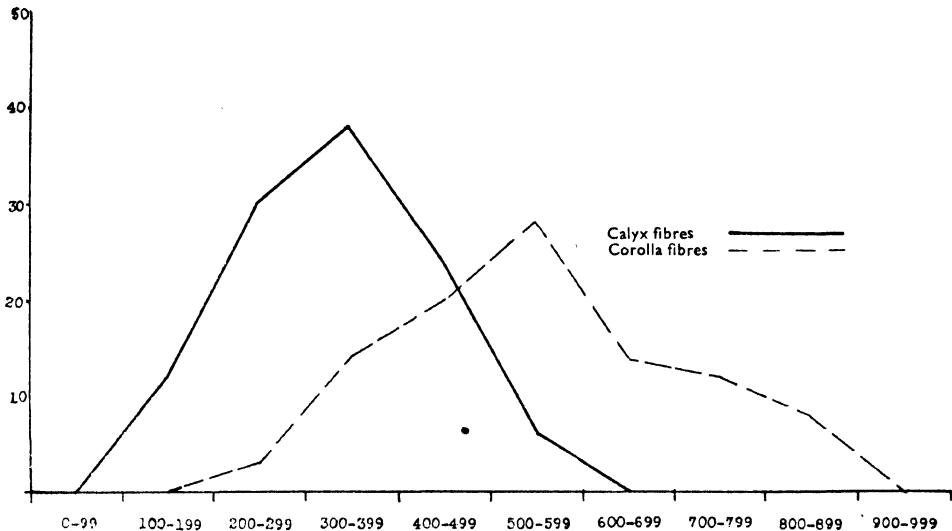


FIG. 3. Dimensional relations of the metaxylary floral fibres of *L. brevidentatus* (Hemsl.) Perk. One hundred fibres were measured for each curve. Vertical numerals refer to the number of fibres for each measurement; horizontal numerals to the length of fibres in micra.

Perhaps the clearest evidence that the fibres of the floral bundles of *Coutoubea* are referable to xylem is obtained from an examination of the junction of the pedicellar stele with that of the peduncle. In a transverse section of that region (Pl. XV, Fig. 6) it is found that the fibres of the pedicel connect directly with the xylem of the peduncle. This fact, together with the absence of pericyclic strands in the peduncle would indicate quite plainly that the floral fibres are wholly derived from the xylem. Exceptions in the flower of *L. brevidentatus* will be discussed in a subsequent paragraph.

In *Coutoubea* the metaxylem is so abundant in the pedicel and receptacle, surrounding the protoxylem in the manner which has just been described, that nodal gaps normally caused by the departure of the calycine and corolline bundles are effectively obscured (Pl. XV, Fig. 5). In the corolla the bundles are not heavily lignified immediately above the receptacle, but somewhat beyond gradually accumulate fibres until the lignification is nearly as heavy as in the calyx (Pl. XV, Fig. 4; Pl. XVI, Fig. 12). Metaxylary elements of the bundles do not pass throughout the entire corolla, however, seldom or sparingly being passed to the branch veins and never towards the lobes. It is probable that the relatively slight lignification towards the base



of the tube may contribute towards a mechanical explanation of the contortion of the persistent corolla about the fruit.

It is somewhat difficult to compare the xylem complements of the floral members with that of the stem in *Coutoubea*, as the latter is characterized by considerable secondary xylem in addition to rather scant metaxylem (Pl. XV, Fig. 6). However, it is evident that the xylary cells present are not as highly specialized as are those of the flower. Normal bicollateral phloem is found in both stem and peduncle. Only normal primary conductive tissues are present in the leaves.

*Lisianthus brevidentatus* (Hemsl.) Perk.

In *L. brevidentatus* metaxylem fibres similar to those of *C. spicata* are found in both calyx and corolla, but in the staminal filaments are found apparently only when those organs are adnate to the corolla tube. The chief distinction of *Lisianthus* in this connexion lies in the frequent occurrence of phloem or pericyclic fibres in addition to the xylary (Pl. XVI, Figs. 9–10). Such elements are considerably greater in diameter and length than the xylary, and are always very conspicuous at the periphery of the bundles containing them. The development of the calycine and corolline bundles of *L. brevidentatus* is essentially similar to that of *Coutoubea*, except that the lignification of the provascular cells originally destined for metaxylem is more irregular, sometimes apparently failing altogether, and giving the mature bundle a jagged, loosely composed aspect (Pl. XVI, Figs. 9–10).

The individual fibrous elements of the metaxylem of *Lisianthus* flowers are much as in *Coutoubea*, except in dimensional relations, and in the fact that they are somewhat less heavily lignified. Those of the calyx range from 137–594  $\mu$  in length and from 7–12  $\mu$  in greatest width; those of the corolla range from 264–858  $\mu$  in length and from 8–12  $\mu$  in greatest width. It is seen from the graphical analysis of the length of calycine and corolline elements provided in Text-fig. 3 that in this instance, contrasting with the situation in *Coutoubea*, the corolline fibres are longer and more variable in length than those of the calyx. The fibres present in the adnate staminal filaments are practically equivalent to those of the corolla tube, as in *Coutoubea*. Comparison of Text-figs. 2 and 3 will show that the calyx fibres of both species are approximately equivalent in both length and variability.

DISCUSSION AND SUMMARY.

The floral bundles of *C. spicata* and *L. brevidentatus* are extraordinary in the large amount of lignified fibres present in the pedicel, calyx, corolla, staminal filaments, and less regularly in the floral bracts. The complete obliteration of the phloem at maturity is also noteworthy. Their interpretation as metaxylary elements finds support, as well in the manner of their

development, in individual structure, and in their relation to the peduncular stele. Their roughly 'mesarch' development is an anomaly in itself.

Mesarch vascular bundles are extremely rare amongst the seed plants. In such instances as the apparently mesarch bundles of the staminal filaments of *Parnassia* (Arber, 1) a compound structure resulting from the fusion of several endarch bundles apparently is obtained. The mesarch bundles of the cotyledons of certain Scitaminae have been interpreted by Miss Berridge (3) as 'due to the relative movement of constituent bundles and therefore of no phylogenetic importance'. The seemingly mesarch orientation of the floral bundles of *Coutoubea* and *Lisianthus* is doubtless to be accounted for upon the basis of secondary orientation as well, since the development in the stem, peduncle, and leaf is endarch. In studies upon the floral anatomy of other gentian flowers, which are yet uncompleted, I have occasionally observed a tendency in the receptacle for certain bundles to form something of a 'horseshoe' position, at times attaining a perfect circle or 'pseudostele' containing a small amount of internal phloem and pith cells, as in *Chelonanthus alatus* (Aubl.) Gilg. In the placental bundles of *Eustoma Russellianum* G. Don infolding of the margins occurs to such an extent that a solid round 'mesarch' bundle is obtained, differing from those of *Coutoubea* and *Lisianthus*, only in smaller size, and in the absence of metaxylary fibres with the associated obliteration of the surrounding phloem.

An examination of Text-figs. 2 and 3 shows that the modes in length of fibres of *C. spicata* are  $350\ \mu$  in the calyx and  $250\ \mu$  in both corolla and staminal filaments; in *L. brevidentatus* the corresponding modes are  $350\ \mu$  in the calyx and  $550\ \mu$  in the corolla. The calycine fibres of both species are therefore equivalent. In *C. spicata* the corolline fibres are shorter and exhibit less variability in length than the calycine. This situation is reversed in the instance of *L. brevidentatus*.

Both of the known species of *Coutoubea* exhibit similar floral fibres. In *Lisianthus*, a large genus which has recently been subjected to much segregation, only the typical element produces the extraordinary fibres. Other gentians which produce similar bundles are species of the genera *Canscora*, *Centaurium*, and *Zygostigma*.

It appears difficult to reconcile the abnormally fibrous condition of the floral bundles of *Coutoubea* and *Lisianthus* with functional needs of the plants, as both are relatively small, herbaceous, outwardly characteristic mesophytes inhabiting the forest borders and moist savannas of the American tropics.

In conclusion, I wish again to express gratitude to my friend Dr. Arthur J. Eames for his continued interest and good council, and to my former students, Dr. M. Elizabeth Pinkerton and Dr. John Adam Moore, who have

assisted me in this investigation under a grant from the Science Research Fund of Washington University (St. Louis) provided by the Rockefeller Foundation.

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#### EXPLANATION OF PLATES XV AND XVI.

Illustrating Dr. Woodson's paper on 'Observations on the Floral Fibres of Certain Gentianaceae'.

##### PLATE XV.

Fig. 1. Portion of the inflorescence of *Coutoubea ramosa* Aubl., showing the lignified floral bundles in the persistent corolla.  $\times 2$ .

Fig. 2. Transverse section of the flower of *C. spicata* Aubl., showing general topography and distribution of fibrous bundles in calyx and corolla tubes.  $\times 23$ .

Fig. 3. 'Mesarch' bundles of calyx, *C. spicata* Aubl., showing extraordinary fibrous development of metaxylem and complete obliteration of phloem.  $\times 215$ .

Fig. 4. Opened dissection of corolla tube of *C. spicata* Aubl., showing the progressive lignification of corolline and staminal bundles.  $\times 8$ .

Fig. 5. Transverse section of lower receptacle of *C. spicata* Aubl., showing extraordinary development of metaxylem which effectively obscures nodal gaps in this region.  $\times 115$ .

Fig. 6. Transverse section of the junction of pedicel and peduncle of *C. spicata* Aubl., showing continuity of the xylem.  $\times 150$ .

##### PLATE XVI.

(All Camera Lucida drawings  $\times 1500$ .)

Fig. 7. Provascular bundle, calyx of *C. spicata* Aubl.

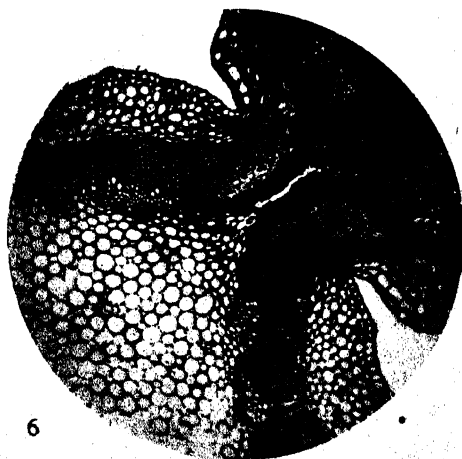
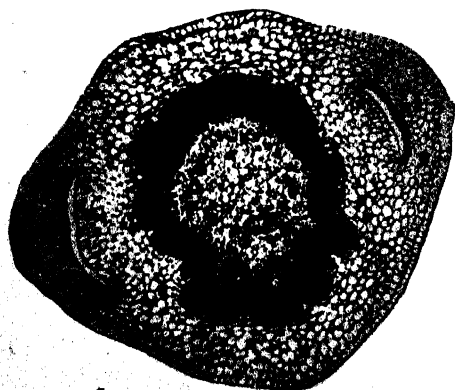
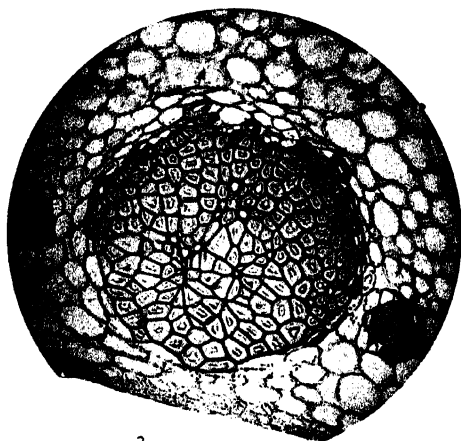
Fig. 8. Development of protoxylem and external protophloem, calyx bundle of *C. spicata* Aubl.

Fig. 9. Young calyx bundle of *Lisianthus brevidentatus* (Hemsl.) Perk., showing irregular development of metaxylem, uncrushed protophloem, and peripheral fibres referable to phloem or pericycle.

Fig. 10. Nearly mature calyx bundle of *L. brevidentatus* (Hemsl.) Perk., showing undifferentiated provascular cells surrounded by metaxylem, crushing of protophloem, and peripheral fibres.

Fig. 11. Mature bundle, calyx of *C. spicata* Aubl., showing 'mesarch' xylem and complete obliteration of protophloem.

Fig. 12. Mature bundle, corolla of *C. spicata* Aubl., showing 'mesarch' xylem and complete obliteration of protophloem.





# The Effect of Carbohydrate and of Nitrogen Deficiency upon Microsporogenesis and the Development of the Male Gametophyte in the Tomato, *Lycopersicum esculentum* Mill.<sup>1</sup>

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With Plates XVII-XIX

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## INTRODUCTION.

SPOROGENESIS and development of the male and female gametophytes in plants have until recently been considered almost entirely subject to genotypic control. That factors in the environment may induce profound changes in these processes has, in general, been given relatively little attention. The recent statement of Beadle (2) in connexion with *Zea mays* that the 'processes of microsporogenesis and pollen development apparently

<sup>1</sup> The work reported herein was very largely carried out while the author was holding a National Research Council Fellowship in the Biological Sciences at the John Innes Horticultural Institution, London, England. To this Institution the author expresses his deep appreciation, not only for the use of its facilities but also for the counsel and assistance of its staff.

depend upon easily disturbed physiological conditions' indicates the swing of the pendulum in the direction of environmental influence. If the physiological mechanism is so easily disturbed it seems logical to expect that environmental factors which alter the physiology of a plant will indirectly produce certain responses once considered subject to genotypic control. Thus pollen and egg cell sterility, flower abscission, and possibly even evolutionary tendencies, might be affected by this alteration in the physiological mechanism.

#### LITERATURE.

There is very little information concerning the effect of specific environmental factors, other than that of temperature, upon sporogenesis and development of the gametophyte. Some years ago East (17) suggested that the capacity of pollen grains to function is probably affected by the environment, and Tischler (65) listed the influences which up until 1928 had been shown to produce disturbances in such processes. Tischler made no attempt, however, to distinguish between the various influences, since under 'climate' he enumerated the work of Stow (59) on temperature, and under 'nutrition' the results obtained by Belling (4) with *Uvularia* and by Jaretzky (22) with *Rumex*, all assumed to be the effects of temperature. Under 'light' the only type of work listed was that with X-rays and radium.

That extremes of temperature may affect the regularity of meiosis and the development of gametophytes has been shown by a number of investigators among whom Borgenstam (7), Sakamura and Stow (47), Stow (59), Michaelis (34), and Randolph (42) are outstanding. That the attacks of gall mites and red spiders and the ravages of a certain virus disease, as well as needle punctures and intergeneric grafting, may also disrupt sporogenesis and development of the male gametophyte has been recently shown by Kostoff and his associates (Kostoff and Kendall (26, 27, 28), Kostoff (24, 25)). Whether such environmental factors as drought, desiccation, light intensity, length of light period, and nitrogen supply may have similar effects has been given little, if any, attention. It has been stated that 'extreme changes of light' (Kostoff and Kendall (28)) may be important, but there has been no explanation or amplification of this assumption.

#### SCOPE OF THE WORK.

This paper is a report of experiments designed to alter the carbohydrate and nitrogen content of tomato plants and to determine the effect of a deficiency of each upon sporogenesis and development of the male and female gametophytes. Only the results on microsporogenesis and development of the male gametophyte are herein presented. A second paper will show the results with megasporogenesis and development of the female gametophyte.

The carbohydrate and nitrogen contents were altered by varying the amount of nitrogen supplied to plants growing under varying day lengths in summer and in winter, as well as by the addition of supplemental illumination to extend the light period.

#### SPECIFIC TREATMENT OF THE PLANTS AND EXPERIMENTAL PROCEDURE.

The work reported herein was largely carried out in the greenhouses and laboratories of the John Innes Horticultural Institution, London, England, during the period from 10 September 1932 to 1 August 1933. Part of the sand culture experiments on nitrogen deficiency were carried out at the Ohio Agricultural Experiment Station, Wooster, Ohio, during July and August 1934.

Five series of plants based on origin, treatment, and season of planting were used in the experiments. The plants were of the Marhio variety, a selection of Marglobe, segregated for pink fruit colour.

##### *Series I—1 October 1932 to 18 February 1933.*

The plants of Series I were grown from seed, and five pots, each containing one plant, were placed in each of the following media: manure (with a small amount of sand); manure  $\frac{2}{3}$ , loam  $\frac{1}{3}$ ; manure  $\frac{1}{2}$ , loam  $\frac{1}{2}$ ; manure  $\frac{1}{3}$ , loam  $\frac{2}{3}$ ; eleven plants were also potted into loam and given additional illumination with two 500 watt Mazda bulbs from 3.30 p.m. to 9 p.m. daily. Six plants were also potted in washed beach sand and supplied by means of the drip culture method (Shive and Stahl (54)) with a full nutrient solution containing ammonium nitrate as the source of nitrogen. A phosphorus-containing organic fertilizer and lime were added to each of the manure and soil treatments throughout all the experiments.

All of the plants of Series I showed symptoms of carbohydrate deficiency in degrees varying with the medium, season, illumination, and age of the plants. The internodes of the plants were long and thin, supple and succulent. The lower leaves were thin, very dark green, and drooping, and, in addition, the upper ones became much crinkled (Pl. XVII, Fig. 1). The pots containing manure only were set in larger pots containing peat which was watered in place of the manure. This possibly accounts for the fact that these plants became quite succulent, the leaves very dark green, and the internodes not as long as those of the plants in the other treatments. The leaves were malformed and crinkled.

A very large proportion of the early formed flower clusters of the plants in Series I, as well as those in Series II, showed no flowers reaching anthesis. The second cluster of the Series II loam plants receiving the additional illumination was usually the first cluster to contain flowers reaching anthesis (Pl. XVII, Fig. 2). The plants not under illumination showed no flowers



reaching full bloom until at least the third cluster was attained. In both Series I and II the number of flowers reaching anthesis and the location of the cluster containing such flowers seemed to correlate positively with the reserve carbohydrate content of the plants as indicated by the presence and location of starch determined microchemically.

The leaves of the plants were examined microchemically at intervals for the relative abundance of starch and nitrates. The plants not given additional illumination showed tremendous accumulation of nitrates in their leaves and stems, but no starch during their early growth. The nitrate content gradually decreased during the period of growth, but some nitrates were present in the plants at the end of the experiment. The starch content of the stem gradually increased during the period in which the growth rate decreased.

The temperature of the house in which the plants of Series I and II were grown was not thermostatically controlled, but it was possible because of the mild weather to keep the temperature within a uniform range of 68 to 70° F. by day and only slightly lower at night.

All plants of Series I and II were free from virus disease.

#### *Series II—4 November 1932 to 18 February 1933.*

Plants were potted on 4 November into each of the following media: Manure (with sand), five plants; manure 1/2, loam 1/2, five plants; manure 1/9, loam 8/9, fifteen plants. The plants in manure were watered directly. Those in loam 8/9 were divided further into three lots, one lot receiving additional illumination along with the illuminated plants of Series I, one lot receiving sodium nitrate, while the third lot was left untreated. Five plants were again grown in sand drip culture, and three of these were given additional illumination.

The plants showed the characteristic symptoms of carbohydrate deficiency as enumerated in the discussion of the plants of Series I. The plants in the loam 8/9 with additional illumination were the first to produce flowers that reached anthesis, and some flowers of the second cluster even set fruit. Not a flower of the second cluster of any of the plants in the other treatments reached anthesis.

Material for examination of the meiotic stages was taken in particular from the flowers of the first three clusters of plants of Series I and II which were showing the most extreme carbohydrate deficiency.

#### *Series III—1 May to 1 August 1933.*

A large number of cuttings were taken from certain of the seed-grown plants of Series I and II (Tables I and II) which had showed sterility of all pollen in flowers of the earlier formed clusters. From these cuttings plants were grown which served as the source of cuttings for the plants of

Series III. In this way a uniform, vigorous set of plants was obtained which were placed in the following media: Manure  $\frac{3}{4}$ , loam  $\frac{1}{4}$ ; manure  $\frac{1}{4}$ , loam  $\frac{3}{4}$ ; and sand drip culture. The plants in  $\frac{3}{4}$  manure were divided into two lots, one receiving ammonium sulphate and the other no treatment. The plants in sand drip culture were divided into two lots. One lot was given a full nutrient solution and the other a full nutrient solution minus nitrogen. No additional illumination was given to any plants of Series III and IV. From May to August the length of the daylight period (15 to 16 hours) was practically double that during which the plants of Series I and II were grown.

The plants in the manure and soil treatment, particularly those in manure  $\frac{3}{4}$  grew very vigorously and produced thick, succulent stems. The leaves were very dark green, succulent, and somewhat malformed (Pl. XVII, Fig. 3). All flowers of all clusters of these plants reached anthesis and produced anthers of a normal yellow colour.

The growth-rate of the plants in sand without nitrogen was retarded; the plants became strongly chlorotic and showed the usual external and internal symptoms of nitrogen deficiency. All growth and flower production finally ceased. During the period of nitrogen starvation flowers were removed for the study of sporogenesis and development of the male gametophyte.

The temperature of the house from May to August frequently ranged higher during the day than 70 to 75° F., but in general it was maintained satisfactorily.

Some of the plants in Series III and IV contracted a mild form of virus, but this had little evident effect.

#### *Series IV—1 May to 1 August 1933.*

This series consisted of sixty-five plants grown from seed of a fruit produced by selfing a flower of plant 30 (Series I) grown in loam. The soil media used were the same as those of Series III. The plants in Series IV became even more vigorously vegetative and a darker green than those of Series III (Pl. XVII, Fig. 3). All flowers reached anthesis and produced anthers of a normal yellow colour.

#### *Series V—6 July to 31 August 1934.*

In order to obtain further data concerning the effect of nitrogen deficiency upon sporogenesis and pollen development, tomato plants were placed in sand drip culture in the greenhouse of the Ohio Agricultural Experiment Station at Wooster. Plants were given a full nutrient solution with sodium nitrate as the source of nitrogen and a similar solution without nitrogen in any form. The plants gradually showed the characteristic symptoms of extreme nitrogen deficiency (Pl. XVII, Fig. 4).

## CYTOLOGICAL PROCEDURE.

For the study of sporogenesis and the development of the male gametophyte both single anthers and whole flowers were selected for fixation. These were usually fixed by immersion for a few seconds in Carnoy's fluid followed by either Medium Flemming or La Cour's 2 BE (30). The usual stain was gentian violet, and the cytological procedure outlined by La Cour (30) was followed throughout. Gentian violet and aceto-carmine smears were frequently made.

Germination tests were made of the pollen on a number of media. Knop's solution (one-half strength) with 1 to 2 per cent. agar was usually used, although the addition of 10 to 20 per cent. sucrose to this solution gave good results. Pollen grains were examined on such agar plates both before and after staining with a number of reagents including cotton blue, ruthenium red, resorcin blue, aceto-carmine, safranin, lacmoid, and potassium iodide-iodine. Pollen grains were also examined in glycerine, lactic acid, and balsam in accordance with the suggestions of Ferguson and Coolidge (18). The grains were also examined on the stigmas after staining with several of the above reagents in order to check the accuracy of the germination results on the agar media. Styles were also dissected and stained by various methods.

EFFECT OF CARBOHYDRATE DEFICIENCY UPON MICROSPOROGENESIS  
AND THE DEVELOPMENT OF THE MALE GAMETOPHYTE.*Reserve Carbohydrate the Factor Altered by the Environment.*

That carbohydrate deficiency was induced by the environmental treatments used (except those in the minus nitrogen group) is verified by several lines of evidence. In the first place the microchemical determinations for starch made in the stems and leaves demonstrated such deficiency. Secondly, the characteristic symptoms of carbohydrate deficiency which have already been described were produced in degrees varying with the media, season, and illumination. Thirdly, the quantitative analyses of tomato plants, growing under an environment somewhat similar as regards nitrogen, made by Kraus and Kraybill (29), Nightingale (39), Nightingale, Schermerhorn, and Robbins (40), and Watts (67) support the conclusion that carbohydrate content was the physiological factor altered by the environmental treatments reported herein. The plants grown during the fall and winter (Series I and II) in the high-nitrogen, low-light treatments were highly deficient in starch, particularly during their early growth. The vigorously vegetative spring- and summer-grown plants (Series III and IV) were also deficient in starch, but to a far less degree than those of the earlier series.

*Variation in Number of Flowers reaching Anthesis as a Response to the Different Treatments.*

The number of flowers of a cluster reaching anthesis increased progressively from cluster to cluster with a specific environmental treatment. Furthermore, between comparable flower clusters on plants of the different treatments, a regular and entirely consistent progression occurred.

*First cluster:* The flowers of the first cluster of all but three of the fall- and winter-grown plants (Series I and II) failed to reach anthesis. Two of the plants of Series I whose first cluster produced a flower reaching anthesis were grown in sand culture and had been slightly retarded in growth by transplanting. Clear-cut differences in the relative size of these flowers which failed to reach anthesis were noted in Series II between the plants grown in the four treatments: sand alone, sand and additional light, soil alone, and soil with additional light (Pl. XVII, Fig. 2). In the plants in *sand or soil without additional light* it was clearly evident that the flowers ceased development at a smaller size than in the corresponding plants receiving additional light. In fact one flower of the first cluster of a plant in soil with additional light had reached anthesis but the pollen was non-germinable.

*Second cluster:* The flowers of the second cluster of the plants of Series I and II were larger in size before their growth ceased (even though they failed to reach anthesis) than the flowers of the first cluster receiving the same light and soil treatment. Pl. XVII, Fig. 2 illustrates the fact that a large proportion of the flowers in Series II in *sand with additional light* attained the normal size of flowers reaching anthesis, but failed to show the separation of petals and sepals. On the other hand, a considerable number of the flowers of the plants growing in *soil with additional light* reached anthesis. In the plants growing in the soil treatments with at least one-third manure, an occasional flower reached anthesis; yet in all but one or two instances this flower contained only sterile pollen. Some of the flowers in *soil alone with additional light* even produced a fair proportion of germinable pollen. None of the flowers of Series II in *sand without additional light* reached anthesis. It is to be noted that the plants of Series II were started about three weeks later than those of Series I, and comparable clusters of the two series thus had developed under different lengths of daylight period.

*Third cluster:* In the third cluster the plants in *loam with additional illumination* had a number of flowers which contained deep yellow anthers at anthesis and set fruit. A much smaller proportion of the plants in one-third manure or more reached anthesis, and these usually contained a very high proportion of sterile pollen (Tables I and II). A similar situation existed between the plants of Series II in *sand with additional*

*illumination* and those of the same series in *sand without additional illumination*.

*Fourth cluster:* By the time of development of the fourth cluster, a few of the plants of Series II in one-third or one-half manure, and some of those of Series I in full manure, produced a number of flowers reaching anthesis, and these had a fair proportion of germinable pollen. The plants of Series II in full manure produced no flowers with normal yellow anthers on the first five clusters and no germinable pollen during the entire period. On the other hand, many clusters of the plants in *soil or sand with additional illumination* produced flowers with deep yellow anthers, showed some germinable pollen, and set fruit rather freely.

It was thus clearly evident that: (1) the additional illumination increased the number of flowers reaching anthesis in the earlier formed clusters and also the germinability of their pollen; (2) the manure treatments reduced, in comparable clusters, the number of flowers reaching anthesis in the early produced clusters; (3) more flowers in comparable clusters on the plants of *Series I* reached anthesis or produced pollen of higher germinability than on the plants of *Series II* due to the longer daylight period under which the plants of Series I were growing; (4) the number of flowers in a cluster reaching anthesis increased with the slowing down of growth which was coincident with the increasing age of the plants.

#### CYTOLOGICAL DATA.

##### *Flowers not reaching meiosis.*

In the flowers of the plants suffering very severe carbohydrate deficiency (those of the smaller sizes as shown in Pl. XVII, Fig. 2) degeneration of the anthers occurred before the development of a distinct sporogenous tissue. In these cases this tissue failed to reach the meiotic divisions and the walls of its cells collapsed and degenerated before the prophase stage was initiated. There was no evidence to indicate that growth cessation of these small flowers was the immediate result of degeneration of sporogenous cells whose cell mechanism was rather delicately balanced from a physiological viewpoint; such degeneration seems rather to have occurred concurrently with a general retardation of the rate of development of the entire flower due to a limitation of carbohydrate material.

##### *Flowers reaching meiosis.*

In the flowers which reached meiosis, the first division in the microsporocytes usually proceeded in a regular manner as far as freedom from lagging and from non-disjunction would indicate (Pl. XVII, Fig. 5). Fig. 6, which shows one metaphase plate with 15 chromosomes and two chromosomal bodies in the outlying cytoplasm, may indicate that the first division did not proceed regularly in that microsporocyte. Observation of many

microsporocytes during the first division showed, however, that lagging as a result of the environmental treatment was rare. It was clearly evident that anaphase and telophase stages when present proceeded in a regular manner. It seemed probable, however, that the growth-rate of the flowers on plants highly deficient in carbohydrates might be so retarded that this division was not always completed. If this cessation occurred, as was occasionally indicated by the staining reaction of the chromosomes and cytoplasm, the division proceeded regularly up to the point where all movement of the chromosomes ceased. In this connexion it was clear that the meiotic stages which usually proceed rapidly in normal plants were long drawn out in the plants highly deficient in carbohydrates. The peculiar physiological state of the cells induced by the environmental treatment used in these experiments thus had a profound effect in regulating the rate of meiosis.

That lagging may be induced in the first division by desiccation was indicated by cytological examination of anthers carefully removed from a single flower at successive intervals. At the beginning of the work it was thought possible to study meiosis by this removal of adjacent anthers of a single flower. It was found, however, that despite great care in the process, the flowers ceased to develop normally thereafter. Apparently the removal of an anther permitted slight desiccation of the remaining anthers, with the result that frequent lagging during the first division was observed. It thus became necessary to discard this method and to fix whole flowers rather than individual anthers unless such anthers were the first to be removed from a flower. This detrimental effect upon the remaining anthers was somewhat similar to that reported by Kostoff and Kendall (28) as a response to needle punctures.

The second division usually proceeded regularly as far as anaphase, even in the plants most deficient in carbohydrates (Pl. XVII, Fig. 7). In a considerable proportion of instances, however, the procession of the chromosomes on one or both spindles was irregular, and the failure of the chromosomal groups to separate completely was observed. In consequence, groups consisting of two more or less intermingled sets of chromosomes which had never fully completed the second division were visible (Pl. XVII, Figs. 8 and 9). In the majority of cases, however, even under severe carbohydrate deficiency, four separate chromosomal groups were formed, even though degeneration often set in before the chromosomes were reconstituted in microspore resting nuclei.

Even under conditions of severe carbohydrate deficiency degeneration of the chromosomal groups occurred most frequently following a second division apparently regular in all respects as far as visual observation would indicate. The number in a tetrad degenerating varied from one to four. Such variation in the number of microspores degenerating per tetrad

correlated positively with the degree of severity of the carbohydrate deficiency. In the flowers of the early formed clusters of the plants of Series I and II growing in one-third or more manure, all four chromosomal groups degenerated at this stage in all or nearly all tetrads (Pl. XVII, Figs. 8 and 9). In these same plants the proportion of functional microspores successively increased in the later-opening clusters as indicated by comparison of these figures with Pl. XVIII, Figs. 13 and 16.

Degeneration of the microspores also occurred almost immediately after their liberation from the tetrad. The cytoplasm and nucleus at first seemed to be closely associated, and the entire degenerating mass held the stain firmly as indicated by Pl. XVIII, Fig. 10. Shortly thereafter both the nucleus and cytoplasm lost their capacity to hold the stain. As a result the early-degenerating microspores seemed to be distinguished at anthesis by their shrunken, irregular shape and by their more or less colourless appearance when stained with gentian violet in fixed preparations (Pl. XVIII, Fig. 11). Furthermore, such microspores at anthesis in degenerated anthers apparently comprised a considerable proportion of the so-called 'pollen grains'. These microspores failed to absorb the aceto-carmin, ruthenium red, or acid fuchsin stains as shown in Pl. XVIII, Fig. 12, and represent practically the fraction characterized as 'visibly aborted grains' in Tables I, II, and III.

In addition to this early degeneration the microspores were subject to degeneration at any period during their maturation up to the mitotic division of the microspore nucleus. This is indicated by their variation in shape, in appearance of their cytoplasm, and in their staining properties, as shown in Pl. XVIII, Figs. 13 to 16. That the division of the microspore nucleus to form the vegetative and generative nuclei did not always proceed regularly in carbohydrate deficient plants can be judged by the situation illustrated in Pl. XVIII, Figs. 17 and 18. It is not possible to state with certainty how the extra chromosomal groups shown had originated, but probably they arose as a result of an irregular division of the microspore nucleus. That they are the result of a premature and irregular division of the generative nucleus seems unlikely, and that they are comparable to the supernumerary nuclei obtained by Moffett (35) in *Kniphofia* seems questionable.

Degeneration of pollen grains was a frequent occurrence. Typically aborted grains are illustrated in Pl. XVIII, Figs. 14 and 15, a single grain of which is shown in Pl. XIX, Fig. 20. Such grains were commonly produced on whole plants suffering from a moderate deficiency of carbohydrate material as well as in flowers of clusters produced under temporary low light conditions which had induced only a localized carbohydrate deficiency of the adjacent stem and leaves. The writer produced such late degeneration by shading the tips of plants with developing flower clusters a few days before the flowers reached anthesis. Such plants had previously pro-

duced germinable pollen grains in all flowers, and the lower parts of the stem axis had considerable starch storage.

Finally, under conditions of less severe carbohydrate deficiency many pollen grains were developed which were morphologically perfect, which took the aceto-carmin or ruthenium red stains deeply, but which failed to germinate on the stigma or the agar media. The proportion of such grains is indicated by the data in Tables I to IV. Thus, although the failure to stain does indicate non-germinability, the fact that the grain is stainable does not insure germination, for the degeneration process may set in during the final stage of development. It is to be noted, for example, that in one instance (Table IV, plant 28, cluster VI, flower 1) only 9.8 per cent. of the pollen grains germinated, but 91.5 per cent. stained in fresh preparations with ruthenium red. The proportion of such grains reached as high as 100 per cent. of the anther contents of some of the last-produced clusters on some of the plants of Series II, and frequently comprised the greater proportion of the non-germinable grains in the plants of Series III and IV (Tables I to III). These grains were usually found at anthesis in anthers of a light to deep yellow colour rather than in anthers of a green to greenish-yellow colour. Occasionally they were also associated with a considerable number of the large type of non-germinable grains to be described subsequently. It is the belief of the writer that these morphologically perfect but non-germinable grains are produced as a response to a mild carbohydrate deficiency set up in a localized area of the stem and leaves contiguous to the flower cluster. Such grains had probably developed in a functional condition up to a brief period just previous to anthesis. A localized deficiency occurring at this time may have also resulted in enzymatic disturbances in the grains which prevented germination. It is not to be understood in this connexion that all the grains in a yellow anther that failed to dehisce normally at anthesis with the production of 'dusty' pollen necessarily contained only non-germinable grains. Often, although the percentage of morphologically perfect but non-germinable grains was fairly high, a considerable number of germinable grains were present. Since in yellow anthers a very high proportion of the grains may also be germinable it is not possible by casual observation of yellow anthers to predict the presence of germinable or non-germinable grains.

In the flowers which reached anthesis, on plants very deficient in carbohydrates, the anthers were usually smaller than normal and a light green in colour. Subsequently they often turned brown and dried up while the pistil was of normal size and colour. Between this green colour and the normal deep yellow colour, anthers with a wide range of green-yellow shades were obtained. Anthers which failed to reach the deep yellow colour almost without exception contained only degenerated microspores and sterile pollen grains (Pl. XVIII, Figs. 12, 14, and 15). Usually a large



TABLE I.

*The Comparative Pollen Germination and Percentage of Visibly Aborted Grains in Plants of Series I and III, Series I—Fall and Winter Plants Grown from Seed; Series III—Plants Produced from Cuttings of Series I Plants.*

Plant and treatment.	Cluster and flower no.*	Visibly aborted grains. %	Pollen germination. %	Corresponding plant and treatment.	Cluster and flower no.*	Visibly aborted grains. %	Pollen germination. %
10 Manure	Series I.				Series III.		
	I	No flowers to anthesis	0.0	10 I Manure 1/4	II 2	8.0	
	II 2	100.0			III 4	9.0	68.5
12 Manure	III 2				III 5	10.0	67.0
	I	No flowers to anthesis			III 4		15.2
	II 4	100.0	0.0	12 1 " 1/4	II 4	12.6	25.3
	III 1	100.0	0.0	12 2 " 1/4	III 2	5.0	95.0
	V 1	98	47.4	12 3 " 3/4	II 1	13.2	63.3
	V 2		4.6	12 4 " 3/4	II 5	3.0	72.8
	V 3		36.1	12 5 " 3/4	III 1	1.3	61.6
				12 6 " 3/4	II 1	5.3	3.4
					III 3	0.0	64.7
					II 3	0.5	88.1
22 Manure 2/3	I-IV	No flowers to anthesis			III 1	0.3	90.0
	V	1 flower reached anthesis			III 2		95.0
				22 7 " 1/4	II 1		50.0
					III 4	2.3	85.6
				22 3 " 3/4	II 1		90.6
				22 4 " 3/4	I 4		85.7
				22 6 " 3/4	III 3	3.9	62.1
				22 3a " 3/4	II 1		50.0

23	Manure 2/3	I-III IV V 1 V 2	No flowers to anthesis 2 fruits developed 0.0 Some germination	23 5 Manure 1/4 23 8 " 3/4 23 2 Sand plus am. nitrate	II 2 III 2 II 1 III 2 III 3	87.1 70.0 87.3 72.6 82.8
28	Loam plus add. light	I-II III IV V 3 VI 1 VI 2 VI 4	No flowers to anthesis 1 flower to anthesis 2 fruits developed 11.1 73.8 9.8 33.0 2.1	28 1 Manure 1/4	I 3 IV 4 IV 5	84.0 83.5 86.5
35	Loam 1/2	I-III IV 2 V 1	No flowers to anthesis 68.0 70.0	35 2 " 1/4 35 4 " 3/4	IV 1 IV 2 IV 3 II 2	90.0 90.0 85.2 77.6
40	Sand plus am. nitrate	I 1 IV 4 IV 5	1.2 0.0 0.0	40 1* " 1/4 40 3 " 3/4 40 10 " 3/4	I 1 II 1 II 1	80.0 89.0 70.0

\* Cluster number in roman numerals refers to order beginning with first cluster on plant. Flower number refers to order of opening of flowers beginning with first to open.

TABLE II.

*The Comparative Pollen Germination and Percentage of Visibly Aborted Grains in Plants of Series II and III. Series II—Fall and Winter Plants Grown from Seed; Series III—Plants Produced from Cuttings of Series I Plants.*

Plant and treatment.	Cluster and flower no.*	Visibly aborted grains. %	Pollen germination. %	Corresponding plant and treatment.	Cluster and flower no.*	Visibly aborted grains. %	Pollen germination. %
Series II.							
68 Manure	I-II	No flowers to anthesis		68 I	Manure 1/4		
	III 1	94.3	0.0		IV 2	0.5	53.7
	III 2	98.0	0.0		IV 3	0.0	78.1
	IV	No flowers to anthesis				3.5	87.9
72 Manure	I-III	No flowers to anthesis		72 I	" 1/4		
	IV 1	No flowers to anthesis	0.0			1.0	86.5
					II 1	0.5	53.7
					II 3	8.0	77.7
					IV 1	0.0	72.3
				72 2	" 1/4	0.0	14.2
				72 3	" 1/4	10.7	91.7
					II 3	2.0	10.0
				72 4	" 3/4	3.0	71.9
				72 5	" 3/4	6.5	81.0
					IV 1	0.0	83.3
				72 6	" 3/4		
					III 2		
73 Manure	I	No flowers to anthesis		73 I	" 1/4		
	II 2	94.2	0.0		II 2	0.9	4.7
	II 3	94.3	0.0		V 1	0.0	89.8
	III 1	100.0	0.0		IV 3	0.0	69.0
	IV 3		0.0	73 6	Sand plus am. nitrate	10.4	56.3
					III 4	0.0	75.5

74 Manure	I	No flowers to anthesis	74 1 Manure 1/4	II 2	1-2	67-6
	II 2	99-7		III 1	0-0	52-3
	II 3	100-0	74 5 " 1/4	I 7	5-1	76-8
	IV 1	0-0		III 1	0-0	18-5
	V 2	0-0	74 6 " 3/4	III 2	4-7	76-7
				II 2		13-5
			74 7 " 3/4	III 1	4-8	33-6
				II 3		14-3
				III 1	1-8	69-4
				III 4	3-0	54-0
			74 10 " 3/4	III 5	4-0	14-6
				IV 1	0-0	27-7
			74 8 Sand plus am. nitrate	II 1	7-2	78-4

\* Cluster number in roman numerals refers to order beginning with first cluster on plant. Flower number refers to order of opening of flowers beginning with first to open.

TABLE III.

*The Comparative Pollen Germination of the Plants of Series IV and the Parent Plant in Series I (Plant 30).  
Plants of Series IV Grown from Seed of One Fruit of Plant 30.*

Plant and treatment.	Cluster and flower no.*	Pollen germination. %	Corresponding plant and treatment.	Cluster and flower no.*	Visibly aborted grains. %	Pollen germination. %
30 Loam plus add. light	Series I.					
	I	No flowers to anthesis	45 Manure 1/4	IV 2	1.7	6.6
	II 3	40.0		IV 3	0.0	80.0
	III	Several fruits developed	4 " 3/4	IV 2	0.0	33.9
	IV	" "	8 " 3/4	IV 3	0.0	90.3
			10 " 3/4	I 2	0.0	14.2
			13 " 3/4	III 4	9.0	68.5
			15 " 3/4	I 2	1.7	4.7
			17 " 3/4	I 4		90.0
			27 " 3/4	I 4		80.0
			36 " 3/4	I 3		90.0
			37 " 3/4	IV 2	0.0	50.4
				III 5	2.3	6.5
				IV 1	0.9	52.6
				V 1	8.4	84.6
			38 " 3/4	IV 4	0.0	76.0
			40 " 3/4	IV 2	1.7	59.8
			61 " 3/4	IV 2	0.0	11.7
			51 Sand plus am. nitrate			
			52 " "	I 3	4.0	63.0
				I 3	31.7	41.6

\* Cluster number in roman numerals refers to order beginning with first cluster on plant. Flower number refers to order of opening of flowers beginning with first to open.

proportion of the contents of such anthers were of the visibly aborted type, being shrunken, malformed, and unable in fresh mounts to hold the aceto-carmine, or ruthenium red stains (Pl. XVIII, Fig. 12).

It is not surprising, therefore, in view of the fact that degeneration occurred at various stages from telophase of the second division up to and

TABLE IV.

*The Relation of Pollen Germination to Percentage of Grains Stained with Aceto-carminc and Ruthenium Red. Series I and II.*

Plant and treatment.	Cluster and flower no.	Grains stained with :		Acid fuchsin. %	Pollen germination. %
		Aceto-carminc. %	Ruthenium red. %		
Series I.					
11 Manure	V 1	76.0			26.6
12 Manure	V 1		90.2		47.4
	V 2	97.3			4.6
	V 3		69.5		36.1
33 Manure 1/2	V 3a*	46.3			0.0
	V 3b*	86.0			0.0
40 Sand plus am. nitrate	IV 4	4.0			0.0
	IV 5	12.3			0.0
28 Loam plus add. light	V 3	94.2	96.1		73.8
	VI 1		91.5		9.8
	VI 2	96.4	96.5	96.6	33.0
	VI 4		78.7		2.1
Series II.					
68 Manure	III 1		5.7		0.0
	III 2		2.0		0.0
73 Manure	II 3		0.7		0.0
	III 1		0.0		0.0
40 Sand plus am. nitrate	IV 4	4.0			0.0
	IV 5	12.3			0.0
51 Loam 8/9 plus add. light	IV 4		82.6		28.4
52 Loam 8/9 plus add. light	IV 2		95.9		71.4
	V 1		95.9		50.0
53 Loam 8/9 plus add. light	V 5		1.6		0.0

\* Adjacent anthers of same flower.

including the mature male gametophyte stage that single locules containing this entire range of degenerated microspores and pollen grains were commonly observed. As indicated in Pl. XVIII, Figs. 14 and 15 degenerated microspores and sterile pollen grains lay side by side within anthers which failed to reach the deep yellow colour characteristic of anthers at normal anthesis. The relative staining properties of the contents of such anthers in fixed preparations (gentian violet stain) is shown in Pl. XVIII, Figs. 14 and 15, and in fresh preparations in Pl. XVIII, Fig. 12. Often a green anther

lay adjacent to anthers of greenish-yellow or yellow colour. The difference in appearance of the contents of adjacent anthers of these types is shown in Pl. XIX, Fig. 21, and the data obtained with stains on fresh material are given in Table IV. For example, in the greenish anthers of Plant 33 (Table IV) only 46 per cent. of the contents of the dark anther stained with aceto-carmin, while in the adjacent light yellow anthers 86 per cent. took the stain. No germination occurred in either case.

*Large non-germinable pollen grains.*

A type of pollen grain with peculiar characteristics was often found to be associated with morphologically perfect germinable and non-germinable grains. Such pollen grains were characterized by their uniformly large size as well as by their peculiar, homogeneous, granular appearance (Pl. XIX, Figs. 22 and 23). These grains failed to take the ruthenium red stain as deeply as did grains of normal size, and the exine seemed to be much thinner. Furthermore, the nucleus was poorly defined and very difficult to stain (Pl. XIX, Fig. 24). Aceto-carmin often seemed to reduce the size of such grains, while ruthenium red had no comparable effect. The grains also appeared to be somewhat similar to the large hypertrophied non-germinable grains obtained by Dudgeon (16) in the degenerating anthers of *Rumex crispus*. The proportion of such grains varied from the tip of the anther to its base, from locule to locule, from flower to flower, and from plant to plant. They were practically non-germinable, and often comprised the greater proportion of non-germinable grains in yellow anthers of a flower suffering from mild carbohydrate deficiency. Such grains also appeared occasionally, but much less frequently, in the nitrogen deficient clusters. Thus it cannot at this time be stated that they are a type produced in response to carbohydrate deficiency alone, but rather as a response to certain environmental treatments conducive to a mild derangement of the physiological mechanism of the cell.

No evidence was obtained in this study to justify the conclusion that such grains were tetraploid. Although their uniformly large size would have supported such a conclusion, their staining properties and non-germinability makes such a conclusion unlikely. To be sure Steere (57) has pointed out that under 'abnormal physiological conditions nearly any plant may be induced to produce diploid or polyploid gametes'. Upon the basis of our present evidence the physiological derangements produced as a result of the environmental treatments used in this study were insufficient to produce that result, important from an evolutionary standpoint.

AMOUNT OF DEGENERATION AND STERILITY CORRELATED WITH  
DEGREE OF CARBOHYDRATE DEFICIENCY.

It was clearly evident throughout the course of this work that the microspore degeneration and pollen sterility correlated positively with the degree of carbohydrate deficiency. Substantiation of this fact is presented by several lines of evidence. In the first place, microspore degeneration and pollen sterility were not a fortuitous, random occurrence but varied concurrently with the severity of the carbohydrate deficiency. As shown in Tables I and II, in the first-formed clusters of the plants of Series I and II, growing under exceptionally high-nitrogen and short-day treatments (manure media) the microspore degeneration was usually 100 per cent. of the anther contents. On the other hand, the flowers of the plants growing in loam soil with additional illumination showed some pollen fertility. Thereupon, with the slowing down of growth which is associated with a lessened organic nitrogen formation, brought about in part by a decreased rate of nitrogen absorption, a proportion of the pollen became fertile even in the manure treatments which originally produced only degenerated microspores. This increase of pollen fertility with flowers of successive clusters took place not only on plants of Series I and II growing under additional illumination (Pl. XVIII, Fig. 19), but also in the flowers of the plants of Series III and IV, which were growing under similar nitrogen treatment but under a longer daylight period with higher light intensity. It is of primary importance to note that the plants of Series III (developed from cuttings taken from the plants of Series I and II which had been grown in manure and had produced only sterile pollen) showed pollen of high fertility even in the first two clusters (Tables I, II, III, Pl. XIX, Figs. 25, 26, 27). Finally, with mild carbohydrate deficiency grains were produced which, though often non-germinable, were morphologically perfect. The later opening clusters of some of the plants of Series I and II had often contained practically nothing but this type of non-germinable grain. With a slight increase in the supply of carbohydrate material such morphologically perfect grains germinated.

## LOCALIZED CARBOHYDRATE DEFICIENCY.

It was noted during this work that microspore degeneration and pollen sterility often varied in the earlier produced clusters of the plants of the various series from flower to flower in the same cluster. Such variation is indicated by certain data in Tables I to IV. Undoubtedly this was due to the localized deficiency set up as a response to variation in the supply of carbohydrates available at a particular time for the development of the sporogenous tissue or the processes involved in microsporogenesis and pollen production. The reserve supply of carbohydrates in these plants at this



time was undoubtedly at a low point, and consequently any variation in the environmental conditions in the direction of reducing the rate of photosynthesis, such as a period of cloudiness extending over several days, variations in light intensity, in water supply, would have a marked effect on the local supply of carbohydrates. With increased carbohydrate reserve in the plant such periodical variations in the environment would have far less effect in producing variations in microspore degeneration and pollen sterility between flowers of the same cluster or of succeeding clusters.

Localized carbohydrate deficiency, as a result of rapid re-utilization of organic nitrogen located in the lower leaves, was also obtained in the plants grown under the nitrogen-deficient treatments carried on in 1934 (Series V). In these plants following the very severe nitrogen-deficient conditions induced, the growth of the stem-tip suddenly commenced and flowers were produced. The stem-tip and upper leaves became the light green characteristic of carbohydrate deficiency and no starch could be found microchemically except in very small amounts in the endodermis. On the other hand, the middle and lower portions of the stem were abundantly filled with starch. The flowers gave evidence of carbohydrate deficiency and the germination of the pollen was at a very low level.

It is thus apparent that localized carbohydrate deficiency can be induced not only by variations in the supply of carbohydrates produced at a particular period in the development of a plant, but also as a result of rapid re-utilization and transfer of organic nitrogen previously held in lower leaf and stem tissues. In the latter case, hindrances to rapid hydrolysis of starch as well as to its translocation to points of use may result in localized carbohydrate deficiency with its unfavourable effects upon microsporogenesis, development of the male gametophyte, and pollen germination.

#### EFFECT OF NITROGEN DEFICIENCY UPON MICROSPOROGENESIS AND THE DEVELOPMENT OF THE MALE GAMETOPHYTE.

##### *Material.*

The plants grown in 1933 at the John Innes Horticultural Institution were obtained from two lots, one grown from seed (plant 30) and the other from cuttings. The cuttings were taken from certain of the fall- and winter-grown plants of Series I and II (plants 23, 73, 74) of which the entire anther contents have shown pollen sterility as a result of the environmental treatments. The plants used in 1934 at the Ohio Agricultural Experiment Station were grown from seed of the Marhio variety.

The plants were put under two treatments, one a complete nutrient solution and the other the same solution containing all essential elements except nitrogen. The plants eventually to receive the minus nitrogen solution were allowed to grow under the complete nutrient treatment for a short period. After transference to the minus nitrogen solution and the plants

had begun to exhibit symptoms of nitrogen deficiency, flowers were removed for cytological examination of microsporogenesis and pollen development. This removal was continued at frequent intervals until growth of the plants ceased and no flowers reached meiosis, a failure which usually occurred at the end of the third cluster (Pl. XVII, Fig. 4). Pollen was also removed during this period for microscopical examination, for staining with various reagents, and for germination tests on an agar medium containing the Knop nutrient solution.

Even under conditions of increasing nitrogen deficiency the flowers usually reached anthesis. This was in marked contrast to the results obtained with carbohydrate-deficient plants, where a large proportion of the flowers failed to reach the normal anthesis even when the deficiency was only moderately severe.

#### CYTOLOGICAL DATA.

Examination of the meiotic divisions in the pollen mother-cells showed that meiosis usually proceeded in a regular manner even under increasingly severe nitrogen-deficient conditions. It was noted, however, that the time consumed in the anaphase and telophase stages was considerably less than that consumed by the same stages in the carbohydrate-deficient plants.

Microsporogenesis in the nitrogen-deficient plants was characterized by a decided regularity as compared to that of the carbohydrate-deficient plants. Even under severe nitrogen-deficient conditions a very large proportion of the microspores and pollen grains was functional (Table V, Figs. 28, 29). The very striking difference in the amount of microspore degeneration and pollen sterility induced by carbohydrate as opposed to nitrogen deficiency is shown by a comparison of the data taken from the carbohydrate-deficient plants of Series I and II (Tables I to III) with those for the nitrogen-deficient plants of Series III, IV, and V (Table V). In particular, note should be taken of the data for the plants of Series I and II and those produced from cuttings taken from these plants (plants 23, 73, 74) and grown under nitrogen-deficient conditions.

Nitrogen deficiency did result in some microspore degeneration and pollen sterility, but sterility of the entire contents of an anther was never obtained in these experiments as a response to treatments inducing such deficiency. It is to be noted in Table V that the percentage of the pollen grains germinating in the flowers of the third and last cluster to reach anthesis varied from 17 to 81 per cent. In comparison, it is to be noted in Table V, from the pollen germination data for the plants receiving the complete nutrient solution that their pollen germination ranged from 41 to 83 per cent. These plants were growing very vigorously, and their physiological condition was similar to that of the other plants of Series III (Tables I and II).

Degeneration of the microspores, when it occurred, took place at the same stages as in the carbohydrate-deficient plants. A smaller number of the large type of non-germinable grains, such as has already been described, (Pl. XIX, Figs. 22-24) was obtained in the nitrogen-deficient plants.

TABLE V.

*The Effect of Nitrogen Deficiency upon Pollen Germination and Percentage of Visibly Aborted Grains.*

Treatment.	Seed plant no.	Clonal plant no.	Cluster and flower no.	Visibly aborted grains. %	Pollen germination. %
Series III and IV—June to July 1933.					
Plus nitrogen	23	2	III 2	0.6	72.6
			III 3	0.0	82.8
Minus „	23	3	III 3	2.3	70.0
Plus „	73	6	III 2	1.1	56.3
			III 4	1.0	75.5
Minus „	73	7	III 4	3.0	54.0
Plus „	74	8	II 1	2.4	78.4
Minus „	74	9	II 5		50.0
Plus „	30-52		I 3	0.9	41.6
Plus „	30-51		I 3	0.0	63.0
Minus „	30-50		I 2	22.7	73.4
			I 3	53.1	26.2
			III 3	0.0	80.0
Series V—July to August 1934.					
Plus nitrogen	8		I 4	25.5	60.8
Plus „	9		III 1		82.5
			III 2	12.1	80.6
Minus „	1		II 5	24.2	70.9
			III 2	8.8	23.3
Minus „	2		I 4	4.0	83.3
			II 1	16.9	53.8
			II 2	21.0	69.3
			III 2	6.0	17.3
Minus „	3		II 1	38.0	53.0
			II 4		81.6
			III 4	9.5	77.7
Minus „	4		II 4	1.0	80.0
Minus „	5		II 4	8.9	36.6
Minus „	6		II 4		84.4

It was thus clearly evident that carbohydrate deficiency resulted in far greater microspore degeneration and pollen sterility than did nitrogen deficiency.

#### DISCUSSION OF THE DATA.

*Substantiation of Work Presented Herein by Other Investigations with the Tomato.*

The results herein presented showing that carbohydrate deficiency in the tomato produced microspore degeneration and pollen sterility are sub-

stantiated by the recent work of Watts (67) who grew tomato plants under a somewhat similar environment. He obtained '100 per cent. abortive pollen' in the anthers which were greenish at anthesis and concluded that the pollen 'seemed to have been arrested in its development after the reduction divisions had taken place'. He made no examination of meiosis and the development of the male gametophyte. Furthermore, Winkler (70) also reported that immature anthers of the tetraploid tomato, *Solanum lycopersicum* *gigas*, during the winter contained empty pollen grains. With better light conditions in the spring more normal pollen grains were produced. Although he presented no cytological data he assumed that degeneration followed a regular reduction division and declared that the irregularities which occurred led to a disappearance of one, several, or all the cells of the tetrad. Sutton and Wilcox (60) reported, as a result of pollen germination tests, that uniformly higher percentages were obtained as the year advanced from January to June. During January they stated that it was very difficult to obtain even satisfactory germination, whereas later in the year long, vigorous tubes were produced on the same medium. They suggest the possibility that 'light and humidity in the greenhouse in which the plants were grown might have affected pollen development'.

*Relation to Work with Plants Other than the Tomato.*

Certain environmental conditions which are primarily characterized by the induction of carbohydrate deficiency have been reported to produce pollen sterility in plants other than the tomato. Winkler (70) also reported that greater pollen sterility was produced in the tetraploid *S. tuberosum* *gigas* under the unfavourable light conditions of spring. Connors (13) pointed out that when the flower primordia of *Dianthus* (carnation) were formed during the dull days of January and February contabescence of anthers, as well as apparently normal but non-germinable pollen grains, was produced. Darrow (15) reported that the stamens in hermaphroditic varieties of *Fragaria* failed to develop normally in darkness and under conditions of low light intensity such as the short days of winter. Although Darrow made no cytological examination, Valteau (66) has presented data to indicate that anther suppression is accompanied by microspore degeneration and pollen sterility. Although de Mol's (36) work on the 'duplication of generative nuclei' in *Hyacinthus* and *Tulipa* is probably largely explained as the effects of temperature, de Mol pointed out that pollen sterility was greater in the diploid varieties of these species when the plants were induced to flower in the greenhouse in January than when they were growing outside under better light conditions. In addition he later (37) concluded that pollen fertility was always higher in plants grown outside than in plants forced in the greenhouse. It would appear from these statements that in these two genera carbohydrate deficiency induced as a consequence of very rapid amino acid

synthesis during a short period of low light intensity was a factor in the production of some pollen sterility. In this connexion Buchholz and Blakeslee (11) have stated that early flowering plants of *Datura* growing in rich soil, as well as the first flowers of vigorously growing plants in the greenhouse show poor pollen fertility. Shull (55) also declared that in shepherd's purse, *Capsella bursa-pastoris*, pollen sterility also developed when the plants were grown under low illumination.

Furthermore, it is occasionally stated in the literature that 'age of plant' and 'changes in nutrition at the time of flowering' (Bleier (6), Poole (41)) are factors associated with pollen sterility. The 'age of plant' explanation is given to the observation that pollen fertility is often less in the first flowers produced by a young, newly flowering plant than in the flowers produced during the mid-blooming period. This was observed by Blaringhem (5) with *Digitalis*, Buchholz and Blakeslee (11) with *Datura*, Cummings and Jenkins (14) and Valteau (66) with *Fragaria*. Poole (41) explained this same observation in the first-formed flowers of four species of *Crepis* as the result of 'changes in nutrition at the time of flowering'. Blaringhem (5) accounted for it in *Digitalis* as the effect of a 'toxic substance' which vanished with the 'maturation and lignification of the tissues'. It is apparent that these explanations of the same external response are concerned with an identical physiological condition in these early produced flowers, namely, carbohydrate deficiency. Such deficiency in the first-formed flowers of plants just coming into bloom is the result of a very rapid synthesis of organic nitrogen coincident with the rapid growth of the plants just preceding flower development. During this period the carbohydrates manufactured are consumed in the synthesis of amino acids and in respiration, thus preventing a sufficient supply to permit the development of functional pollen grains. This situation also developed, it is to be noted, in the first-formed flowers of the tomato plants of Series III and IV (Tables I to III) which were making a vigorously vegetative type of growth. Later on sufficient material of a carbohydrate nature accumulated to permit normal microsporogenesis and pollen development.

That still other species of plants exhibit a decreased pollen fertility as a result of carbohydrate deficiency seems highly probable on the basis of our present evidence. The first step in support of this conclusion lies in the establishment of the fact that anther suppression in plants is preceded or accompanied by reduction of pollen fertility. This is a justifiable premise, as is indicated by the work of Stout (58) with *Cleome*, that of Jaretsky (22) with *Rumex*, and that of Darrow (15) and Valteau (66) with *Fragaria*. Jaretsky (22) found, in fact, that anther suppression in the male and hermaphroditic flowers of *Rumex flexuosus* and *R. maritimus* was accompanied by cytological irregularities in microsporogenesis almost identical with some of those herein reported with *Lycopersicum esculentum*. Furthermore, the

writer (work unpublished) has found that in January and February the pollen in anthers of the abnormally small flowers of *Cucumis sativus* was completely sterile.

With the establishment of the fact that anther suppression is accompanied by decreased pollen fertility, the question arises as to what plants other than those considered above exhibit anther suppression as a result of environmental conditions characterized by the induction of carbohydrate deficiency. Schaffner (52, 53) and Richey and Sprague (44) have shown that in the monoecious *Z. mays* anther suppression is induced by environmental treatments such as light of short duration or low intensity which may be interpreted as principally characterized by the induction of such carbohydrate deficiency.

*Further Evidence of the Carbohydrate Requirement of Microsporogenesis and Development of the Male Gametophyte.*

Still further evidence of the carbohydrate requirement for the development of functional pollen grains is presented indirectly by the results of several investigators. Lidforss (32) stated that weak light was a factor influencing the development of pollen but failed to develop the point further. Tischler (64) listed over one hundred species of plants, which were in nearly all cases characterized by pollen possessed of a high starch content. Upon analysis of his tabular data it becomes apparent that environmental conditions markedly affected the deposition of such material. That differences in starch reserve are likely an important characteristic of functional and non-functional pollen grains of some plant species is indicated by the results of Renner (43), who found such variation in *Oenothera* species as a result of genetic factors, and was apparently of the opinion that the condition was the immediate physiological result of genic variation. Brink (10) reported that in *Z. mays* the sterile grains were devoid of starch and fat. Bregger (9) used the iodine test for starch as an index of pollen fertility in the sugar cane. Rudloff and Schmidt (46), in considering the effect of unfavourable weather conditions on the reduction division in *Oenothera* species mention 'normal grains filled with starch and abnormal grains lacking starch'. It would thus appear that the presence of starch which indicates a carbohydrate reserve is associated with the production of fertile pollen grains.

To be sure, Whyte (69) in discussing the failure of pollen development in a number of plants growing under similar environments has suggested that pollen sterility was due to the coincident development of both the male and female organs and the inability of the male to compete with the female in obtaining the requisite amount of food materials. He failed, however, to take into account that in such plants microsporogenesis and

development of the male gametophyte usually preceded megasporogenesis and development of the female gametophyte and degeneration of the tetrad occurred prior to any development on the female side beyond the megasporocyte stage. In fact, support of the conclusion that there exists a particular requirement of the male organs for carbohydrate material is given by the recent results of Bouillene, Bouillene, and Ghene (8), who report that the soluble carbohydrates were higher in the male than in the female flowers of *Mercurialis perennis*. Furthermore, not only was the total photosynthesizing leaf surface higher in the males, but the consumption of sugar was much greater during the formation and development of the male flowers. They suggested the necessity for the expenditure of much energy in the formation of pollen grains.

That the failure of pollen to germinate when it is morphologically perfect may be due in part to low activity of certain enzymes effecting changes in carbohydrates is not unlikely. In this connexion Tischler (63) has reported that in *Cassia fistula* sterility of the morphologically perfect pollen grains was due to diastase deficiency. It is not unreasonable to presume that enzymatic activity is a factor not only in the germination of the pollen, but also in the growth of the pollen tubes through the stylar tissue.

#### *Effect of Nitrogen Deficiency upon Microsporogenesis and Development of the Male Gametophyte.*

On the other hand, in *Lycopersicum esculentum*, even under severe nitrogen-deficient conditions, a high proportion of the pollen grains was germinable. The conclusion that nitrogen deficiency as compared with carbohydrate deficiency has relatively little effect upon anther development, and pollen fertility is supported indirectly by the observations of a number of investigators. Smith (56) with *Solanum melongena* var. *esculentum*, Darrow (15) with *Fragaria*, and Stout (58) and Murneek (38) with *Cleome*. Although these workers did not study microsporogenesis and development of the male gametophyte, their results show that the anthers were not suppressed in development as a consequence of environmental conditions which induced nitrogen deficiency. In fact, Murneek (38) stated that the outstanding result of his work with *Cleome* was the normal development of the anthers despite nitrogen deficiency. Wentworth (68) showed a very high pollen fertility in diploid varieties of *Pyrus Malus* growing under conditions where nitrogen was the principal limiting factor. As pointed out by Smith (56) plants of *S. melongena* var. *esculentum* in a nitrogen-deficient soil exhibited much pistil suppression. The writer found (work unpublished) that the pollen in flowers of plants whose pistils were suppressed germinated over 78 per cent.

*Significance of the Different Response of the Microspores and Male Gametophytes to Carbohydrate and to Nitrogen Deficiency.*

The full significance of the different response of the microspores and the male gametophytes to carbohydrate as opposed to nitrogen deficiency cannot be grasped without consideration of the response of the female elements to the same deficiencies. In a following paper data will be presented to show that the latter are relatively little affected by carbohydrate deficiency but are decidedly repressed by nitrogen deficiency. Support of this conclusion is given by the writer's cytological observations on the female sex mechanism (not yet completed), and is substantiated by the observations of Smith (56) with *S. melongena* var. *esculentum*, Darrow (15) with *Fragaria*, and Murneek (38) with *Cleome*. Furthermore, horticultural practice, as well as pollination experiments, affirm the conclusion that nitrogen deficiency commonly induces the derangement of the female sex mechanism in seed-bearing plants.

When the reverse response of the male and female organs to nitrogen and to carbohydrate deficiency is analysed it becomes evident that ultimately we are dealing with the larger problem of the environmental factors which result in sex suppression. *Carbohydrate deficiency* has thus resulted in the repression of the male organs (or, as is sometimes stated, has induced 'femaleness') in such common hermaphroditic plants as *L. esculentum*, *S. tuberosum* *gigas*, *Fragaria*, *Dianthus*, *S. melongena* var. *esculentum* and *Datura stramonium*; and in the monoecious species *Z. mays* and *Cucumis sativus*. On the other hand, *nitrogen deficiency* has resulted in the suppression of the corresponding female organs in these plants.

That the different effect of carbohydrate and of nitrogen deficiency upon the male and female organs is not confined to such hermaphroditic and monoecious species is indicated by the results of several investigators. Gardner (19) concluded in listing a number of changes in the sex expression in plants that 'a low carbohydrate content is associated with the production of femaleness in some monoecious and dioecious species'. Halstead (21) reported that heavy manuring in *Cannabis sativa* increased the proportion of female plants, and Schaffner (50) reported also that low light intensity resulted in the suppression of the male organs in male plants in the same species. Atkinson (1) has reported earlier that in *Arisaema triphyllum* male and neuter plants transferred to a rich soil changed to female in one year. Later Schaffner (50) concluded, as a result of considerable work on sex expression and sex reversal in a number of plant species, that the 'reversal of sex is probably brought about by a physiological condition dependent in some way on the carbohydrate-nitrogen ratio in the cells'. His work with *A. triphyllum* L. Torr., *Cannabis sativa* L., and *Morus alba* L. are examples of those species from which he



drew this conclusion (51). Sansome (48) also agreed that 'light and the balance of reserve products to mineral salts' are important environmental factors in controlling sex expression in these and other plants. The work of Bouillene, Bouillene, and Ghenne (8) with *Mercurialis perennis* already cited confirms the conclusion that the development of the male organs and the male sex expression in some dioecious species is associated with the presence of a relatively high carbohydrate content. Recently, Talley (61) working under Loehwing's direction, presented data indicating that in *Cannabis sativa* a higher soluble and insoluble carbohydrate content is characteristic of the male plants and a higher nitrogen (much lower carbohydrate) content of the female plants.

Thus it is apparent that the male sex expression in a number of plant species is associated with a relatively high content of materials of a carbohydrate nature and the reduction of the content below a certain minimum required for the functional development of the male organs resulted in the suppression of these organs, in microspore degeneration, and in pollen sterility. On the other hand, the female sex expression appeared to be associated with a relatively high organic nitrogen content and the reduction of this content below a certain minimum required for the functional development of these female organs resulted in their suppression. Since carbohydrate deficiency may occur very readily as a response to environmental conditions favouring a very rapid synthesis of organic nitrogen from inorganic nitrogen it is readily understood why extremely rapid synthesis (which is commonly associated with a rapid, vigorously vegetative type of growth) is conducive to the suppression of the male sex organs. Under such conditions the female sex mechanism appears to develop with comparatively little hindrance. On the other hand, environmental conditions conducive to little synthesis of organic nitrogen (with its accompanying carbohydrate accumulation) are responsible for a repression of the female sex organs and elements but have comparatively little effect upon male sex expression.

Because of this and other differences in the materials required for the complete development of the male and female sex organs statements have arisen in the literature pertaining to the specific 'metabolic rate' favouring the development of each (Riddle, 45). Sansome (48) stated that the 'rule holds that the female organs are situated in a position of higher metabolic rate than the male organs'. It has also been stated that the female organs develop 'under a higher level of nutrition' than the male organs (Atkinson, 1; Geddes and Thompson, 20). Meehan (33) stated that in *Picea Abies*, *Corylus*, *Carpinus*, *Quercus*, *Juglans*, and *Alnus*, female flowers are found on the strongest growth, while with 'a weakened vitality there is an increased power to bear male flowers and that only with the highest conditions of vegetative vigour are female flowers produced'. Levitzky

(31) has stated that the male side in *Veratrum* has developed where there has been a definite 'lack of nutritive material'. These statements when taken together obviously refer to the observation that the female condition is associated with (is not repressed by, as is the male condition) a vigorously vegetative type of growth which from a physiological viewpoint is characterized by a mild carbohydrate deficiency. On the other hand, the male organs are relatively little suppressed by poor growth induced by insufficient organic nitrogen—a condition, however, which decidedly limits the female sex expression. It is interesting to note in this discussion of the relationship of metabolism to sex that although Riddle (45) states that the Manoilov reaction, as used by Satina and Blakeslee (49) is a better indication of the metabolic rate than of sex, he concludes that it is of value because of the primary relation between metabolism and sex in plants.

There is further evidence to indicate that there exist other differences in the physiological mechanism of the male and female sex organs than the nitrogen-carbohydrate relationship discussed in this paper. This assumes that these differences are interrelated in ways that will be indicated by further research. In this connexion attention should be called to the work of Bouillene, Bouillene, and Ghenne (8), to that of Joyet-Lavergne (23) on the oxidation-reduction potential of the sexes of plants and that by Talley (61) whose work has already been cited. Joyet-Lavergne (23) reported that the polarized cells of the male plants and organs had an rH greater than the polarized cells of the female, and explained the Manoilov reaction on the fact that reduction was thus greater in the female plants and organs than in the male. Bouillene, Bouillene, and Ghenne (8) also showed that the male plants of *Bryonia dioica* have a greater oxidative capacity than the female. Riddle (45) concludes that the Manoilov test as used in animals and plants really suggests a higher oxidation rate in the male and greater storage in the female of certain substances more easily oxidized than the molecules of Dahlia substance supplied in the test. Finally, it is interesting to note that Camp (12) has shown that the catalase activity of the male parts of a number of those plants used by Schaffner (50, 51) is higher than that of the corresponding female structures. This is of interest because of the probable relation of catalase activity to oxidation and respiration.

#### *Relation of the results to pollen taxonomic studies.*

Obviously, if pollen sterility may be so readily produced by environmental conditions inducing carbohydrate deficiency, care must be taken in interpreting the data concerned with pollen sterility and pollen germination in certain plant species grown under varying environmental conditions. Viability tests of pollen taken from plants grown in the field in summer, in the greenhouse in January, and in very rich soil in spring may be of little value in determining the effect of such genetic influences as male-sterile

factors, and lethal factors upon pollen development. In fact, Belling (3) declared that examination of many flowers is desirable, and from several seasons in succession if one is to determine the precise sterility due to genotypic factors. Poole (41) concluded that some distinction should be made between pollen germination counts taken during the first two weeks of flowering in *Crepis* and those taken later. Although it is not assumed by the writer that all heterosporous plant species show such decided effects of carbohydrate deficiency as are shown by *L. esculentum*, yet the possibility of such a reaction must be taken into account in any study concerned with the effect of male-sterile and lethal factors and cytoplasmic inheritance upon pollen sterility if the plants used in such studies have been grown under varying environmental conditions.

*Relation of the results to flower abscission and fruit and seed development.*

Carbohydrate deficiency when sufficient to result in sterility of all pollen in the anthers of a flower will obviously become the decisive factor regulating the abscission of such flowers, if fruit and seed development are dependent on self-pollination. In *L. esculentum* the effect of pollen sterility upon flower abscission will be considered in a later publication. Abscission due to the same cause may be expected to develop in the other plant species which have been shown to exhibit pollen sterility as a result of similar environmental factors. In *Fragaria* such pollen sterility would be conducive to poor achene development, while in *Datura*, *Buchholz* and *Blakeslee* (11) attribute difficulty in fruit setting in part to poor pollen germination. On the other hand, in plants whose flowers are normally cross-pollinated pollen sterility induced by carbohydrate deficiency would be of little importance in fruit and seed development provided a source of viable pollen were available on adjacent plants.

SUMMARY.

This paper is a report of experiments designed to determine the effect of a deficiency of carbohydrate and of nitrogen upon meiosis in the pollen mother-cells, and upon the development of the male gametophyte in *L. esculentum* Mill. The carbohydrate and nitrogen contents of the plants were altered by varying day lengths, and by supplemental illumination, to extend the light period.

*General results on anthesis and pollen sterility.*

1. The additional illumination increased the number of flowers of the earlier-formed clusters on the fall- and winter-grown plants reaching anthesis as well as the germinability of the pollen.

2. The manure treatments reduced the number of flowers in comparable clusters reaching anthesis, and induced sterility of all pollen in the flowers of the early formed clusters.

3. Because of the longer daylight period, more flowers of the plants grown during the fall reached anthesis or produced pollen of high germinability than was the case with comparable clusters on the winter-grown plants.

4. The number of flowers reaching anthesis increased with the slowing down of the growth which was coincident with the increasing age of the plants.

*Cytological Data.*

*Carbohydrate deficiency.*

1. In the anthers of the flowers of plants suffering very severe carbohydrate deficiency the sporogenous tissue failed to reach the meiotic divisions.

2. In the anthers of the flowers whose sporogenous tissue reached meiosis the first division usually proceeded in a regular manner.

3. In the second division in a small percentage of instances lagging of the chromosomes occurred during anaphase with the subsequent failure of the chromosomal groups to separate completely on one or both spindles. In the majority of cases, however, even under severe carbohydrate deficiency, four separate chromosomal groups were formed, even though degeneration set in before the groups were reconstituted as microspore resting nuclei.

4. Degeneration of the chromosomal groups occurred most frequently following a regular second division.

5. The number of microspores in a tetrad degenerating varied from one to four. Such variation correlated positively with the degree of severity of the carbohydrate deficiency. In the flowers of the early formed clusters of the plants grown during the fall and winter, all four groups degenerated at this stage in all the tetrads of most anthers. In these same plants the proportion of functional microspores successively increased in the flowers of the later-opening clusters.

6. Degeneration of the microspores also occurred almost immediately following their liberation from the tetrad.

7. In addition to this early degeneration, the microspores degenerated during their maturation up to the mitotic division of the microspore nucleus.

8. Frequently the division of the microspore nucleus proceeded irregularly, as indicated by the extra chromosomal groups in pollen grains.

9. Degeneration of mature pollen grains was also a frequent occurrence. Usually such grains contained only two distinct nuclei, but frequently pollen grains with extra chromosomal groups were apparent.

10. Finally, under conditions of mild carbohydrate deficiency many pollen grains developed which were morphologically perfect, and yet failed to germinate either on the stigma or on the agar media. The proportion of such grains reached 100 per cent. of the anther contents of some of the last clusters formed by the fall- and winter-grown plants, and frequently

comprised the greater proportion of the total number of grains in vigorously vegetative plants grown during the spring and summer.

11. The anthers which were of subnormal size and were not of the deep yellow colour characteristic of anthers at anthesis contained only degenerated microspores and sterile pollen grains. On the other hand, anthers of normal yellow colour frequently contained a very high proportion of the non-germinable but morphologically perfect pollen grains.

#### *Nitrogen deficiency.*

1. The meiotic divisions proceeded with their usual regularity in the anthers of flowers on plants grown in a nutrient solution containing all essential elements but nitrogen, and which showed the characteristic internal and external symptoms of nitrogen deficiency.

2. Furthermore, as directly opposed to the carbohydrate-deficient plants, microsporogenesis and the development of the male gametophyte proceeded with a high degree of regularity. Even in the last anthers to reach microsporogenesis and the mature male gametophyte stage in the nitrogen-deficient plants, a very large proportion of the microspores and pollen grains was functional.

3. Nitrogen deficiency when very severe did result in some microspore degeneration and pollen sterility, but even in the last cluster (the third cluster usually) developed on plants severely nitrogen-deficient, 17 to 81 per cent. of the pollen grains germinated. The pollen grains on comparable plants grown in a full nutrient solution ranged from 41 to 83 per cent. germination.

4. Degeneration of the microspores and pollen sterility occurred at the same stages in the nitrogen-deficient plants as in those suffering from carbohydrate deficiency. Furthermore, some morphologically perfect but non-functional pollen grains were also obtained.

#### *General Conclusions.*

1. *Carbohydrate deficiency* resulted in the suppression of the male organs and the production of microspore degeneration and pollen sterility in *L. esculentum* Mill. From an analysis of the literature it is observed that a similar response appears to have occurred in certain hermaphroditic plants as *S. tuberosum*, *Fragaria*, *Cleome*, *Dianthus*, and *Datura stramonium*, and in the monoecious species *Z. mays* and *Cucumis sativus*. A similar response to carbohydrate deficiency probably occurred in certain dioecious species as *Arisaema triphyllum* (L.) Schott, *Cannabis sativa* L., and *Morus alba*, L.

2. Nitrogen deficiency, on the other hand, in the experiments reported herein, affected microsporogenesis and the development of the male gametophyte relatively little. The literature also indicates that the male organs

of the hermaphroditic, monoecious, and dioecious plants listed above were relatively little affected by conditions primarily characterized by the induction of nitrogen deficiency.

3. This differing response of the microspores and pollen grains to *carbohydrate* as opposed to *nitrogen* deficiency is concerned ultimately with the large problem of sex suppression and 'sex reversal' in plants.

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## EXPLANATION OF PLATES XVII TO XIX.

Illustrating Dr. Freeman S. Howlett's paper on 'The Effect of Carbohydrate and of Nitrogen Deficiency upon Microsporogenesis and Development of the Male Gametophyte in the Tomato, *Lycopersicum esculentum* Mill'.

### PLATE XVII.

Fig. 1. Carbohydrate-deficient plant of Series I in manure 1/2, 13 October 1932. Note long internodes, spindly stems, crinkly leaves, and flowers failing to reach anthesis.

Fig. 2. Size of typical flowers of first and second clusters of plants grown under the treatments of Series II. Note the progressive increase in size of the flowers from the plants in sand with no additional illumination to those in soil with additional illumination; note also increase in size from first to second cluster in respective treatments.

Fig. 3. Vigorously vegetative plants characteristic of type of growth of plants of Series III and IV. June-July 1933.

Fig. 4. Characteristic type of growth of plants growing in complete nutrient solution (left) and those in nutrient solution lacking nitrogen (right). Series V. July-August 1934. After three flower clusters had been produced no further growth was made by the nitrogen-deficient plants.

Fig. 5. Regular telophase of Division I in flower of first cluster of plant (75) in manure. Series II. 9 December 1932.  $\times 2,400$ .



Fig. 6. Irregular metaphase of Division II in flower of first cluster of plant (70) in manure. Series II. 10 December 1932.  $\times 1,200$ .

Fig. 7. Regular telophase of Division II in flower of first cluster of same plant shown in Fig. 6 (plant 70).  $\times 2,400$ .

Fig. 8. Members of the tetrad degenerating before reconstitution as microspore resting nuclei. Flower of second cluster of plant in manure (72). Series II. 28 January 1933.  $\times 1,200$ .

Fig. 9. Tetrads showing degeneration of many microspores in flower of same cluster of plant (72) shown in Fig. 8.  $\times 600$ .

#### PLATE XVIII.

Fig. 10. Degenerate microspores in flower of fourth cluster of plant (71) in manure. Series II. 24 January 1933.  $\times 275$ .

Fig. 11. Microspores in green anthers which had degenerated shortly after liberation from tetrad.  $\times 600$ . Note that these microspores failed to hold the gentian violet stain.

Fig. 12. Degenerate microspores and visibly aborted pollen grains on agar media. Only 6 grains in field took the stain ruthenium red. From green anthers of flower of fourth cluster of plant grown in sand supplied with full nutrient solution (plant 40). 22 January 1933.  $\times 80$ .

Fig. 13. Degenerate and functional microspores in flower of fifth cluster of same plant shown in Figs. 8 and 9. Series II. Manure. 1 February 1933.  $\times 475$ . Note that now possibly 50 per cent. of microspores are functional.

Fig. 14. Locules of green anthers of flower of third cluster of plant (73) grown in manure, containing only degenerate microspores and sterile pollen grains. The fresh preparation of these locules stained with ruthenium red appeared similar to that in Fig. 12. Series II. 25 January 1933.  $\times 165$ .

Fig. 15. Degenerate microspores and sterile pollen grains in green anthers of flower of fourth cluster grown in loam soil with additional illumination. Plant 27. Series I. 18 December 1932.  $\times 475$ . Degeneration in this flower did not occur until male gametophyte had developed.

Fig. 16. Functional and degenerate microspores in flower of fifth cluster of plant (68) grown in manure. Series II. 28 January 1933.  $\times 475$ . Note that now nearly 50 per cent. of the microspores are functional.

Fig. 17. Sterile pollen-grain from locule illustrated in Fig. 14. Plant 73. Series II—manure. 25 January 1933.  $\times 840$ .

Fig. 18. Sterile pollen grains found in green anthers of flower of fourth cluster of plant (9) grown in manure. Series I. 2 January 1933.  $\times 840$ . Note several chromosomal groups in certain grains.

Fig. 19. Functional and degenerate microspores in flower of fifth cluster of plant (54) in loam with additional illumination. Series II. 28 January 1933.  $\times 475$ . Note that no more than 30 per cent. of the microspores are now degenerate. Compare with Fig. 16.

#### PLATE XIX.

Fig. 20. Sterile pollen grains of type commonly found in flower of sixth cluster of plant (73) grown in manure. Series II. 11 February 1933.  $\times 1,900$ .

Fig. 21. Dark (left) and yellowish (right) anthers adjacent to one another in same flower, both showing degeneration, but at different stages. From flower of fourth cluster of plant (51) in loam with additional illumination. Series II. 1 February 1933.  $\times 165$ .

Fig. 22. Large non-germinable type of pollen grain interspersed among normal germinable grains. Stained with ruthenium red. Series IV. 7 July 1933.  $\times 80$ .

Fig. 23. Similar grains on agar (unstained). Taken from plant 30. Series I.  $\times 80$ .

Fig. 24. Large non-germinable type of pollen grain in fixed preparation stained with gentian violet. Plant of Series II. 8 July 1933.  $\times 475$ .

Fig. 25. Functional and degenerate microspores in flower of clonal plant of Series III propagated from seed-plant 12 of Series I. 8 June 1933.  $\times 475$ . The darker stained microspores are possibly degenerating.

Fig. 26. Functional pollen grains in flower of same clonal plant shown in previous figure.  $\times 475$ .

Fig. 27. Germinating pollen grains from flower of clonal plant of Series III propagated from seed-plant 72 of Series II. 21 June 1933.  $\times 80$ . Compare with Figs. 8 and 9 taken from seed-plant 72.

Fig. 28. Section of locule taken from flower of clonal plant of Series III propagated from seed-plant 73 of Series II showing functional microspores despite severe nitrogen deficiency of plant. 16 July 1933.  $\times 325$ .

Fig. 29. Germinated pollen grains taken from flower of plant of Series IV grown under severe nitrogen deficiency. 21 June 1933.  $\times 80$ .



# The Germination of Seeds of *Potamogeton*.

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With eleven Figures in the Text.

THE genus *Potamogeton* is the largest and most important group of true aquatic phanerogams inhabiting shallow ponds and lakes in the northern United States and Canada. Most *Potamogetons* propagate vegetatively very freely either by tubers, winter buds, or creeping rootstocks. Some species mature fruits in large numbers; others fruit in abundance only occasionally or under certain conditions; a few have been observed only rarely, if ever, with mature fruits.

## I. HISTORICAL.

Recorded observations of the occurrence of *Potamogeton* seedlings in their natural habitat appear to be rare in literature. The few records of seedlings obtained by germinating seeds are widely scattered and are mostly limited to a few of the larger species.

Irmisch (10) germinated and illustrated a few seedlings of *Potamogeton natans*. Sauvageau (19) recorded seedlings of *P. crispus*, *P. perfoliatus*, and *P. pectinatus* obtained within less than one year after the seeds were placed in laboratory cultures. Seeds of *P. natans* remained dormant for three to four years before germinating. Esenbeck (4) illustrated seedlings of *P. coloratus* and stated that the seeds germinate easily if kept for some time in stagnant water. Moore (14) succeeded in germinating a small percentage of over-wintered seeds of *P. americanus* and *P. pectinatus*.

Guppy (7) planted seeds of several species in water and in wet mud. *P. obtusifolius* and *P. lucens* continued to germinate for five years; *P. pusillus* and *P. natans* continued to germinate for four years; *P. crispus*, *P. oblongus*, and *P. densus* germinated after having been buried for two years. Seeds taken from the stomachs of wild ducks all germinated in a short time. Seeds of *P. natans* that had been fed to a domestic duck showed 60 per cent. germination the following spring. Guppy (7) seems to have been the first to investigate the effect of drying upon the viability of seeds of *Potamogeton*.

<sup>1</sup> I am indebted to my colleague, Professor K. M. Wiegand, for valuable suggestions in the preparation of this paper. Mr. R. T. Clausen and Mr. Otis Curtis, Jr., assisted with the harvesting of the seeds used in the germination tests.

He stated that seeds of *P. natans* germinated freely after drying for four months, but all rotted after being dried for thirty months. Those of *P. densus* germinated after drying for eleven weeks, but all rotted after drying for seventeen and thirty months. On the other hand, the fruits of *P. crispus* germinated in numbers after drying for eighteen months. He concluded that delayed germination was a characteristic feature of seeds of Potamogeton and that they retain their power of germination for several months after drying.

Fischer (6) kept seeds of *P. lucens* and *P. pectinatus* and other aquatics in culture for several years without germinating; but if fermentation was allowed to set in germination soon began. He concluded that seeds of aquatic plants possess dormant embryos and that a chemical stimulus, which under natural conditions is brought about by fermentation and decay of organic matter in ponds, is necessary to bring about germination.

Crocker (2) found that seeds of *P. pectinatus* and *P. natans* gathered green and put into distilled water at 23° C., which was repeatedly changed, gave a considerable percentage of germination, while seeds allowed to ripen in cold water of ponds and kept at 23° C. or 29° C. did not germinate. When ripe seeds were sterilized and the coats ruptured he obtained 51 per cent. and 53 per cent. germination in a water bath at 29° C. as against no germination in sterilized unruptured seeds. Crocker pointed out that in the seeds of these aquatic plants the nature of the seed-coat (pericarp), limiting or excluding water or oxygen supply, may have more to do with inhibition of germination than the nature of the embryo, as was assumed by Fischer (6).

McAtee (13), in his discussion of the propagation of wild duck foods, stated that *P. pectinatus* can be grown easily from seeds. He mentioned that Mr. Dwight Lydell of Michigan State Fish Hatchery had succeeded in propagating it from seeds. McAtee advised mowing the plants when the seeds are mature and scattering them at once in the water where they are to be grown or else placing them in water in cold storage until spring. Pirnie (17) stated that, although germination tests have been made with a few species of Potamogeton, very little information is available to indicate what success might reasonably be expected after planting seed. Maguire and F. B. Wann (12) found large numbers of *P. pectinatus* seedlings growing on a mucky bottom in the Bear River Bird Refuge in Utah. Here they also found, on 22 April 1933, large numbers of seeds washed upon the shore. The seeds that had dried out were non-viable, but those that were still in the water germinated in abundance when placed in water cultures in the laboratory.

The infrequent development of mature fruits and the apparent absence of seedlings in nature, on the one hand, and the very efficient vegetative propagation on the other hand, have been made the basis for theoretical discussions concerning certain aspects of reproduction in several subsections of

Potamogeton. These discussions are based upon the assumption that in some species which regularly propagate by winter buds, this is the usual, if not the only, means of vegetative reproduction.

The need for information on the germination of seeds of Potamogeton may be illustrated by quoting from the introduction to that very comprehensive taxonomic treatment of the linear-leaved North American species of Potamogeton by the most critical American student of this group, Professor Fernald (5).

‘In such heavily fruiting species as *Potamogeton zosteriformis*, *P. Friesii*, *P. strictifolius*, *P. obtusifolius*, *P. pusillus*, and *P. Vaseyi* one would expect, naturally, to find that many, if not most, of the plants spring from seeds; but so generally is the old winter-bud found at the base of the ascending stem that we are forced to the conclusion that, in the species which regularly produce winter buds, these hardened and abbreviated branch tips are the usual, if not the only, means of reproduction.

‘The point here raised, that freely fruiting members of the *Compressi*, *Pusilli* and *Javanici* seem to propagate chiefly, if not solely, by winter-buds, should be experimentally checked. Herbarium specimens by the thousand and the best illustrations indicate that their reproduction is wholly vegetative. If the fruits of these species, often produced in great abundance, are, indeed, not viable, the fact is of great significance. At present no experimental evidence on this point is available.’

In brief, the main points of Professor Fernald’s discussion of the problem may be summed up as follows. Many of the Potamogetons, especially in the subsection Pusilli, rarely produce seeds. It is even doubtful whether some ever produce viable seeds. Wind pollination is assumed, but the conditions under which these plants grow often make wind pollination difficult and seed production highly uncertain. The bases of the plants show no evidence that they came from seeds, but almost invariably have the remains of winter buds attached, indicating that they were produced by vegetative propagation.

## II. MATERIALS AND METHODS.

Several years ago investigations were begun with the hope of obtaining some of the evidence on the viability of seeds of Potamogeton, the lack of which had been pointed out by Fernald (5). For two years repeated attempts to induce the seeds of several species of Potamogeton to germinate, by various methods, all met with failure when the seeds were allowed to become dry before they were placed under conditions favourable for germination. It had been pointed out by Duvel (3) that seeds of wild rice, *Zizania aquatica*, if allowed to dry out seldom germinated; if the seeds dropped into the water or were stored in cold water they retained their viability until the following spring, or even longer. On the assumption that seeds of Potamogeton,

which normally fall into the water when they are shed by the plant, should not be harmed by storage in water, beginning in 1933, a part of every lot of seed intended for germination experiments was stored in water as soon as it was harvested.

*Species tested.* Seeds were obtained from 21 species representing most of the twenty subsections of the genus. In the following outline are listed the species used in the germination tests. Their position within the genus is indicated according to Fernald's treatment (5).

*Potamogeton—Pondweeds.*

- I. Subgenus—*Coleogeton*
  - A. Sect. *Connati*  
Subsect. *Filiformes*—*P. filiformis* Pers.
  - B. Sect. *Convoluti*  
Subsect. *Vaginati*  
    ,, *Pectinati*—*P. pectinatus* L.
- II. Subgenus—*Eupotamogeton*
  - C. Sect. *Adnati*
  - D. Sect. *Axillares*  
Subsect. *Crispi*—*P. crispus* L.  
    ,, *Monticoli*—*P. confervoides* Reichenb.  
    ,, *Compressi*—*P. zosteriformis* Fernald  
    ,, *Oxyphylli*  
    ,, *Pusilli*  
Series *P. connati*—*P. foliosus* Raf. var. *macellus* Fernald  
                            *P. panormitanus* Biv.  
Series *P. convoluti*—*P. obtusifolius* Mert. & Koch  
                            *P. pusillus* L.  
    ,, *Javanici*—*P. Vaseyi* Robbins  
    ,, *Hybridi*—*P. capillaceus* Poiret  
                    *P. spirillus* Tuckerm.  
    ,, *Nutalliani*—*P. epihydrus* Raf.  
    ,, *Alpini*  
    ,, *Amplifolii*—*P. amplifolius* Tuckerm.  
    ,, *Colorati*  
    ,, *Nodosi*—*P. americanus* C. & S.  
    ,, *Natantes*—*P. natans* L.  
    ,, *Lucentes*—*P. angustifolius* Berch. & Presl.  
                    *P. gramineus* L. var. *graminifolius* Fries  
    ,, *Praelongi*—*P. praelongus* Wulf.  
    ,, *Perfoliati*—*P. Richardsonii* (Benn.) Rydb.  
                    *P. bupleuroides* Fernald.

*Harvesting of seed.* Seeds<sup>1</sup> were harvested from *Potamogeton* plants growing in the lakes and ponds of New York State between 20 August and 12 September 1932, 1933, 1934, 1935. To avoid confusing the different species, only seeds still attached to the plants were taken. An herbarium

<sup>1</sup> The term seed, as here used, refers to the mature carpels which in *Potamogeton* in the fresh condition are one-seeded small drupe-like fruits which look like seeds. Actually the coiled seed is surrounded by a hard endocarp and pulpy exocarp. Upon drying these fruits usually appear like nutlets.

specimen was taken at the same time when seeds were harvested. The inflorescences with attached seeds were placed in wet cloth bags until field head-quarters were reached. There each lot was placed in a two-quart glass jar containing at least twice as much water, by volume, as seeds. After the seeds were taken to Ithaca they were stored in diffuse light in a laboratory. Leaves, broken inflorescences, immature seeds, small snail shells, and other extraneous materials were separated from the apparently sound seeds.

*Storage of seed.* After the seeds were washed several times in water, they were placed in clean tap water in two-quart jars and stored in a dark cold-storage room at a temperature of  $1^{\circ}\text{C}.$  to  $3^{\circ}\text{C}.$  This storage in cold water approximates the conditions under which the seeds exist under natural conditions when they fall to the bottom of a pond. Each lot was covered by about twenty times its volume of water and protected against evaporation. The water was not changed during the cold-storage period. The seeds entered cold storage between September 18th and September 23rd, and remained there for ten to fifty weeks or until it was desired to test them for germination. The treatment thus outlined insured a supply of seed which had never been dry, even on the surface, nor was it allowed to become heated by fermentation processes. For comparison, similar lots of seeds were prepared, air dried, and stored in manila envelopes in a dark cupboard in a laboratory.

The seeds were divided into seven series and stored in the dark as follows:

Series 1. Stored dry for two to three months at room temperature; germination test started 10 Nov. 1933.

Series 2. Stored dry for five to six months at room temperature; germination test started 6 March 1934.

Series 3. Stored dry for one year at room temperature; germination test started 18 Sept. 1934.

Series 4. Stored in tap water for two to three months at room temperature; germination test started 10 Nov. 1933.

Series 5. Stored in tap water for two to three months at  $1-3^{\circ}\text{C}.$ ; germination test started 10 Nov. 1933.

Series 6. Stored in tap water for five to six months at  $1-3^{\circ}\text{C}.$ ; germination test started 6 March 1934.

Series 7. Stored in tap water for one year at  $1-3^{\circ}\text{C}.$ ; germination test started 18 Sept. 1934.

*Germination tests.* At the end of the storage period the seeds were placed under conditions favourable for germination. For each test 200 seeds were placed in glass jars and covered with tap water 20-30 cm. deep. The jars were placed in diffuse light on a bench in a glass-house until germination ceased, or for a period of eight months. The cultures were examined daily and about once a week all germinated seeds or seedlings



were counted and removed. Some were transferred to larger culture jars for further observation; others were preserved for morphological studies. Cultures which showed no signs of germination after eight months were placed in cold storage again for two months before re-testing to determine whether the failure to germinate was due to dormancy or death of the embryo.

### III. DATA.

The results of the germination tests are taken up by series. The germination percentages for the different treatments are summarized in the Table.

Series 1 and 2. Seeds which had been air dried for two to six months failed to germinate.

Series 3. Out of twenty species which had been stored dry at room temperature for one year, only two species showed any germination, *Potamogeton americanus* 1 per cent. and *P. gramineus* var. *graminifolius* 0.5 per cent. After the seeds had been in the 'germinators' for eight months without germination, one lot of seeds of each species was cut open so as to expose the embryo and then again placed in 'germinators'. Another lot of each species was placed in cold storage at 1–3° C. for eight weeks and subsequently placed in 'germinators'. The seeds failed to germinate with either treatment. An examination of the embryos showed that they were soft and water soaked.

The failure of the seeds in series 1, 2, and 3 to germinate appears to have been due to the fact that after drying most of them were dead and not dormant.

Series 4. Out of eighteen species stored in water at room temperature previous to the test, only seven showed any germination. *P. gramineus*, *P. natans*, and *P. capillaceus* showed only 1, 2, and 6 per cent. germination respectively. *P. obtusifolius*, *P. conservedes*, *P. angustifolius*, and *P. foliosus* showed better germination, 16, 18, 31, and 60 per cent. respectively. Seeds of *P. foliosus* and *P. obtusifolius* showed some germination within two months from the time when they were harvested, during the time that the seeds were stored in the laboratory preparatory to the germination test. Most of the other species in this series were slow to germinate, only one or two seeds germinating at a time.

Series 5. Seeds of eighteen species were stored in water at 1–3° C. for two to three months, and then placed in 'germinators'. Germination was obtained in all species, very low in several species, but good in others (see Table). The low temperature during the period of cold storage increased the percentage of germination even in those species which germinated best in series 4, stored in water in the laboratory.

Series 6. The seeds of the seventeen species which had been stored in water at 1–3° C. for five to six months all gave higher percentages of germination than those stored for a shorter time. While some germination was obtained from every species used, the percentages were rather low in some,

but above 80 per cent. in seven species, *P. americanus*, *P. angustifolius*, *P. capillaceus*, *P. confervoides*, *P. epihydrus*, *P. foliosus*, and *P. obtusifolius*. The germination percentages obtained for the various species cannot be taken as an indication of the relative fertility of their seeds. The percentages indicate the performance of particular lots of seeds of a species when

*Table Showing the Percentage of Seed Germination in Several Species of Potamogeton.*

Species.	Stored dry.			Stored in water.			
	2-3	5-6	1	2-3	2-3	5-6	1
	months.	months.	year.	months.	months.	months.	year.
	Series	Series	Series	Room temp. Series	1-3° C. Series	1-3° C. Series	1-3° C. Series
	1.	2.	3.	4.	5.	6.	7.
<i>P. americanus</i>	0	0	1	0	65	86	77*
<i>P. amplifolius</i>	0	0	0	0	31	47	—
<i>P. angustifolius</i>	0	0	0	31	78	92	74*
<i>P. bupleuroides</i>	0	0	—	0	6	27	—
<i>P. capillaceus</i>	0	0	0	6	31	94	91*
<i>P. confervoides</i>	0	0	0	18	47	86	71*
<i>P. crispus</i>	0	0	0	—	—	—	—
<i>P. epihydrus</i>	0	0	0	0	47	82	78*
<i>P. filiformis</i>	0	0	0	—	—	—	—
<i>P. foliosus</i>	0	0	0	60	86	89	81*
<i>P. gramineus</i>	0	0	0.5	1	6	37	49
<i>P. natans</i>	0	0	0	2	7	28	39
<i>P. obtusifolius</i>	0	0	0	16	78	81	76*
<i>P. panormitanus</i>	0	0	0	0	4	12	—
<i>P. pectinatus</i>	0	0	0	0	17	38	—
<i>P. praelongus</i>	0	0	0	0	18	—	—
<i>P. pusillus</i>	0	0	0	0	21	57	40
<i>P. Richardsonii</i>	0	0	0	0	9	19	—
<i>P. spirillus</i>	0	0	0	0	6	18	—
<i>P. Vaseyi</i>	0	0	0	—	—	—	—
<i>P. zosteriformis</i>	0	0	0	0	5	16	—

\* Some seeds germinated during storage.

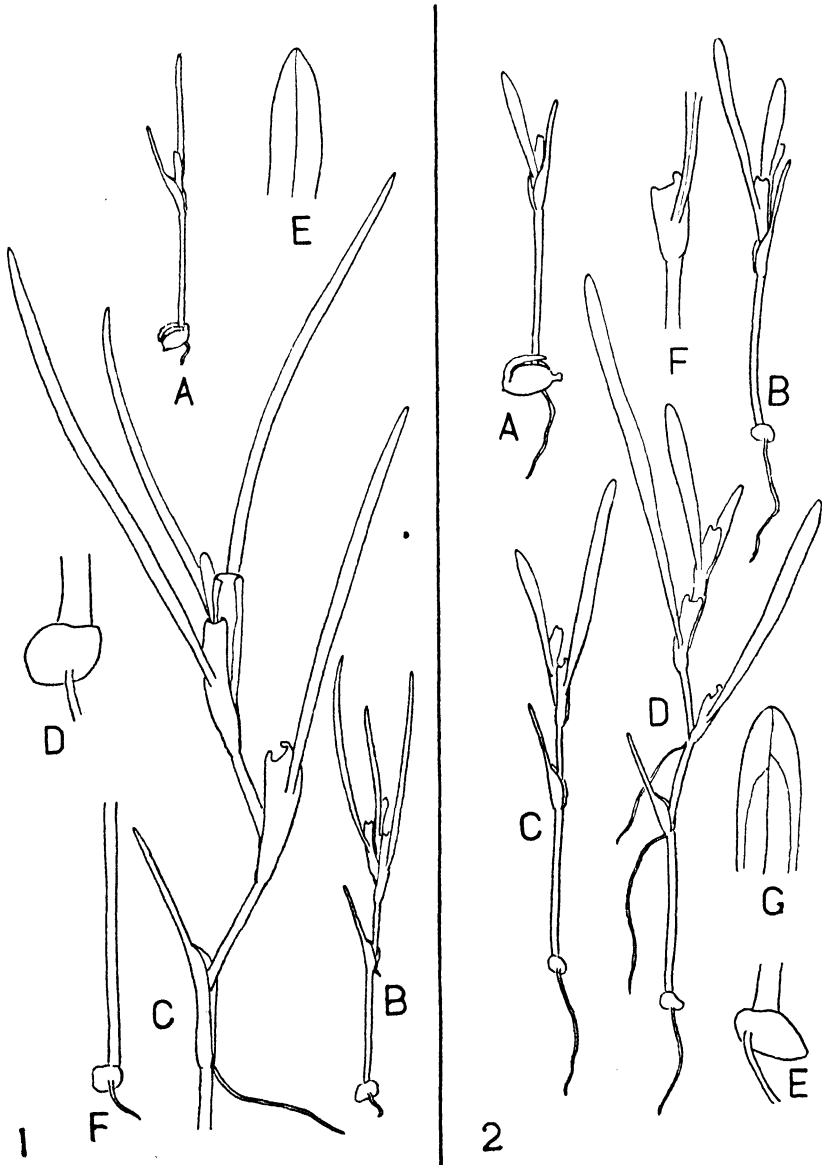
subjected to the conditions indicated. The fact that above 80 per cent. germination was obtained for seven species indicates that, in general, the treatment applied was favourable for their germination. The lower germination in the other species tested may have been due to one or more of several factors. It has been stated before that the seeds had to be harvested while they were still attached to the plants. Some of the seeds so harvested may have been unripe. It was not practicable to determine whether all the seeds used had mature embryos. In some samples a few, or many, of the seeds, may have had immature embryos or none at all. There is also a possibility that some of the treatments applied to the seeds between the time of harvest and their entrance into cold storage may have been injurious to some of the embryos.

Series 7. Seeds of ten species were left in cold storage, 1–3° C., for one year and then placed in the glass-house. All species still had some viable seeds, but those samples which had the highest percentage of germination after five to six months in cold storage had a slightly lower percentage of germination after the longer period of storage. In most of the species (see Table) which remained in cold storage longer than six months some seeds, sometimes as many as 30 per cent., germinated before they were transferred to the glass-house. The ability of these seeds to germinate in water at 1–3° C. at once suggests the conditions under which they occur in nature. The writer has found seeds and seedlings of *Potamogeton obtusifolius* and *Najas flexilis*, in various stages of germination, on the bottom mud in a shallow pond near Ithaca, N. Y., in late November under 5–6 cm. of ice. It seems probable that in their natural habitat, many *Potamogeton* seeds germinate in very early spring or even in late autumn. The germination percentages recorded in the Table include those seeds which had germinated while still in cold storage.

#### IV. DISCUSSION.

In discussing the results of the germination tests it will be necessary to recall briefly some of the conditions under which different kinds of *Potamogetons* produce flowers, disperse their pollen, and develop fruits. According to most authors (1, 5, 8, 9, 11, 18), the species of the subgenus *Eupotamogeton* are wind pollinated, while those in the subgenus *Coleogeton* are water pollinated. *Coleogeton* includes such species as *P. pectinatus*, *P. filiformis* and *P. vaginatus*. The two former produce fruits in abundance and also propagate vegetatively, mostly by subterranean tubers, less frequently by axillary buds, and also by creeping rootstocks. *P. vaginatus*, under New York conditions, very rarely matures fruits but propagates itself extensively by tubers and rootstocks. *Eupotamogeton* includes the section *Adnati*, with the North American *P. Robinsii*, which apparently matures fruit only very rarely (15) but maintains itself by vegetative propagation, and the section *Axillares* for which Fernald records sixteen subsections for North America, including three general types of species: 1. Large forms, often with dilated 'floating leaves' which buoy up their comparatively large flower spikes above the surface of the water; these fruit in abundance, propagate by creeping rootstocks and sometimes by winter buds. 2. Smaller forms, with narrow submerged leaves and broad 'floating leaves'; these also fruit abundantly and may or may not propagate by winter buds. 3. Small forms with very narrow or linear leaves and falsely terminal or axillary spikes which are too easily submerged to allow for sufficient wind pollination to ensure abundant fruits. Species in this group propagate freely by winter buds which are considered an adaptation to conditions unfavourable for fruit formation dependent upon wind pollination.

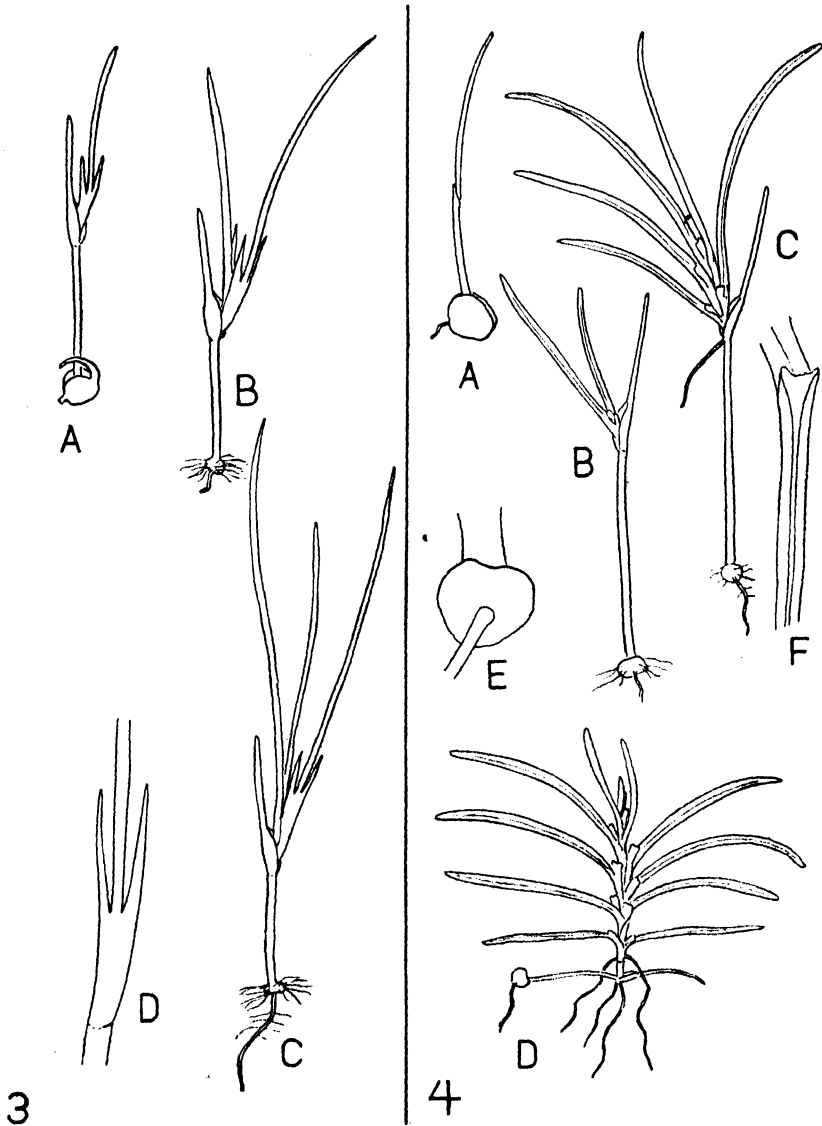
Out of twenty-one species of *Potamogeton* on which germination tests were made, with air-dry seeds stored for from two to twelve months practically



FIGS. 1 and 2. 1. *Potamogeton foliosus*. A and B, seedling, 3 and 12 days after germination;  $\times 2$ . C, seedling with secondary root, 20 days;  $\times 4$ . D, 'foot' enlarged;  $\times 12$ . E, apex of first leaf;  $\times 12$ . F, basal part of hypocotyl;  $\times 4$ . 2. *Potamogeton obtusifolius*. A, B, and C, seedling 5, 10, and 20 days after germination;  $\times 2$ . D, seedling showing secondary roots, 38 days;  $\times 2$ . E, base of first leaf with sheathing stipules;  $\times 4$ . G, apex of leaf;  $\times 12$ .

no germination was obtained. If only dry seeds had been used one might have gained the impression that *Potamogeton* seeds had a low viability. On

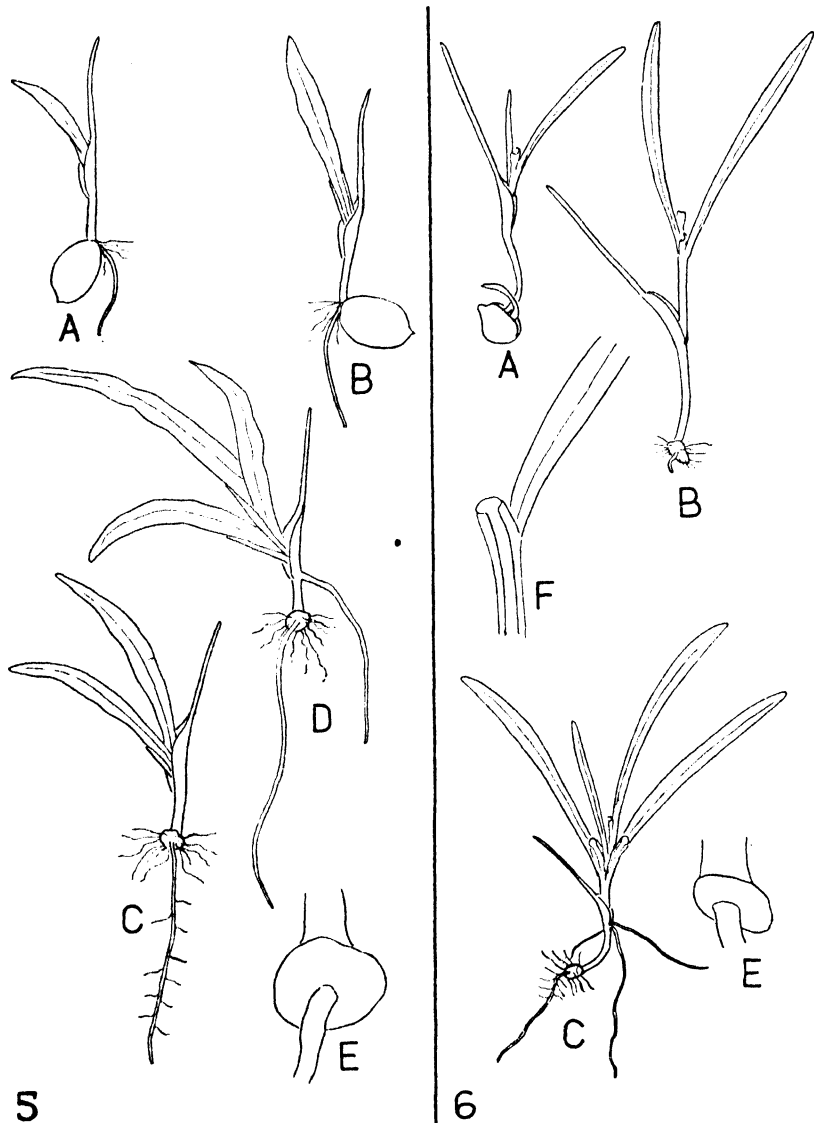
the other hand, with seeds that had never been allowed to air dry between the time of harvest and the germination tests, out of eighteen species tested,



FIGS. 3 and 4. 3. *Potamogeton confervoides*. A, B, and C, seedlings 4, 8, and 15 days after germination;  $\times 2$ . D, base of leaf with stipules;  $\times 4$ . 4. *Potamogeton epiphydrus*. A and B, seedlings 3 and 7 days after germination;  $\times 2$ . C, seedling with secondary root, 18 days;  $\times 2$ . D, seedling developing into a short axis with crowded leaves resembling a winter bud, 38 days after germination;  $\times 1$ . E, base of hypocotyl with 'foot';  $\times 12$ . F, leaf base showing sheathing stipules;  $\times 5$ .

some germination was obtained in every one (Figs. 1-10). The percentages of germination were rather low in some but good (above 80 per cent.) in seven species belonging to six different subsections of *Eupotamogeton*,

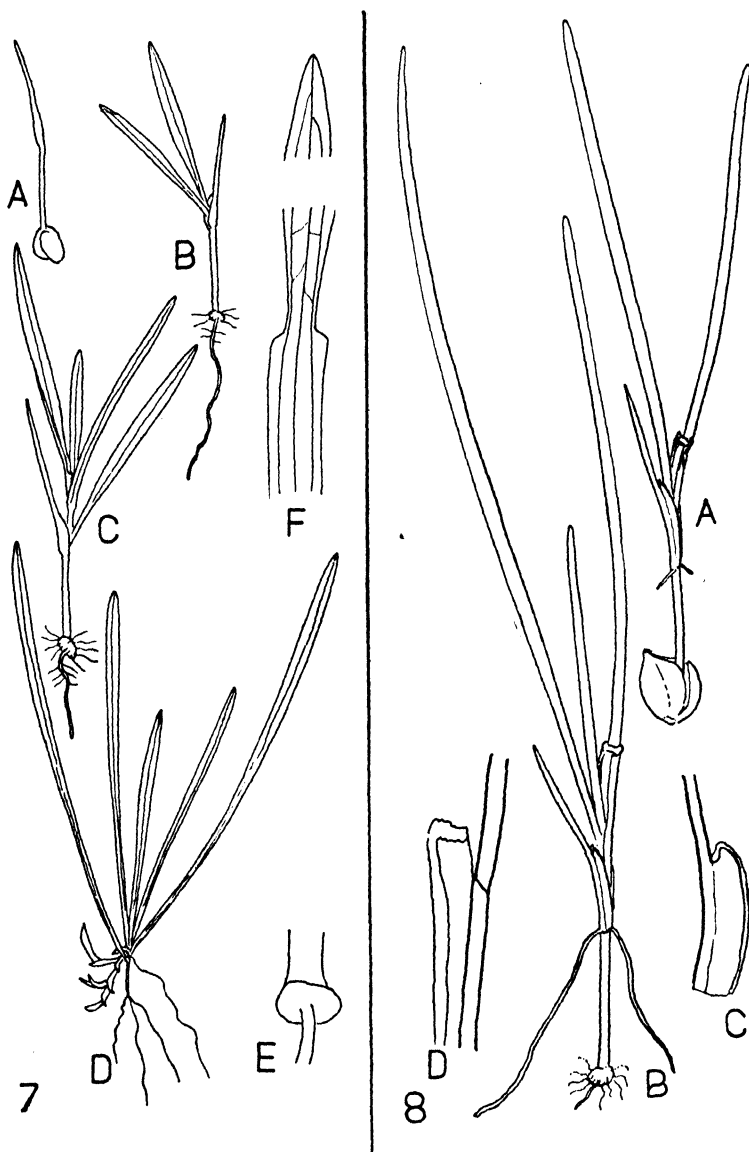
i.e., Monticoli, Pusilli, Hybridi, Nuttalliani, Nodosi, and Lucentes. In the subsection Pusilli, in which propagation by winter buds is common, and in



FIGS. 5 and 6. 5. *Potamogeton amplifolius*. A, B, and C, seedlings, 3, 8, and 16 days after germination;  $\times 2$ . D, seedling showing both primary and secondary roots, 32 days after germination;  $\times 2$ . E, base of hypocotyl showing 'foot';  $\times 12$ . 6. *Potamogeton angustifolius*. A and B, seedling, 10 and 18 days after germination;  $\times 2$ . C, seedling with primary and secondary roots, 38 days after germination;  $\times 1$ .

which conditions for wind pollination are frequently very unfavourable, the four species tested, *P. pusillus*, *P. foliosus*, *P. obtusifolius*, and *P. panormitanus*, all gave some germination, proving that all these species are still

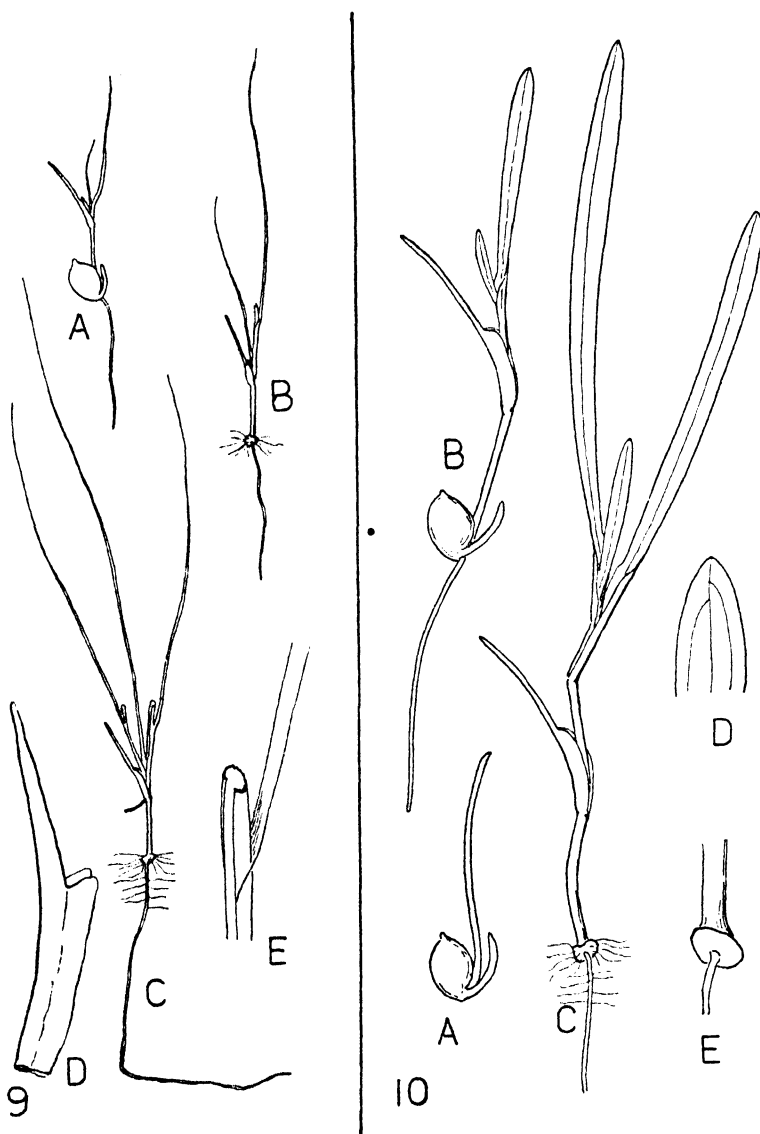
capable of producing viable seeds. The demonstration of the fact that they can yet produce by seeds of course does violence to the theory that vegeta-



FIGS. 7 and 8. 7. *Potamogeton americanus*. A, B, and C, seedlings 3, 9, and 20 days after germination;  $\times 2$ . D, seedling showing secondary roots and beginning of rhizomes, 56 days after germination;  $\times 1/2$ . E, base of hypocotyl;  $\times 6$ . F, base and apex of first leaf;  $\times 3$ . 8. *Potamogeton natans*. A, seedling 20 days after germination;  $\times 1$ . B, seedling showing secondary roots, 38 days;  $\times 1$ . C, base of cotyledon;  $\times 3$ . D, base of first leaf, showing sheathing stipules;  $\times 3$ .

tive propagation is a response to the condition under which wind pollination, and subsequently seed production, is nearly impossible.

Coleogeton, which is presumably water pollinated, contains species which fruit in abundance, *P. pectinatus*, and also species which rarely fruit



FIGS. 9 and 10. 9. *Potamogeton capillaceus*. A, B, and C, seedling 4, 10, and 28 days after germination;  $\times 2$ . D, cotyledon;  $\times 10$ . E, base of first leaf showing stipular sheath;  $\times 10$ . 10. *Potamogeton gramineus*. A, B, and C, seedlings 4, 26, and 45 days after germination;  $\times 4$ . D, apex of first leaf;  $\times 12$ . E, base of hypocotyl with 'foot';  $\times 12$ .

under certain conditions, *P. vaginatus*. Both species have highly developed methods of vegetative reproduction, regardless of whether or not they produce fruits. In *Eupotamogeton* vegetative propagation, by one means or



another, is common in most species and in nearly every subsection, whether its members produce fruits freely or not. The results of the germination tests here presented indicate that the forms with a highly developed vegetative propagation have not lost the ability to produce viable seeds.

It has already been pointed out by Pearsall (16), Fernald (5), and others, that certain Potamogetons may fail to fruit for many years, but during a season of extreme heat and drouth when the water-level becomes unusually low, may fruit freely. It cannot be assumed, as had been done by some, that the fruiting induced by lowering the water was necessarily due to a change which increased the chances for wind pollination. Lowering the water-level may modify various other factors such as temperature, light intensity, rate of photosynthesis, and growth, any of which may increase the possibility of fruit production.

Those who have presumed that all species of Eupotamogeton are wind pollinated have argued that, since some species, especially Pusilli, grow under conditions which prevent dispersal of pollen by wind, they cannot produce seed. In the first place, there is no evidence at hand to indicate that all species of Eupotamogeton are wind pollinated, but there is at least strong circumstantial evidence that some species of Pusilli and Hybridi produce seeds without wind pollination. It has been pointed out (1, 8), and can be verified by field observations, that *P. spirillus*, even in shallow water, in addition to the terminal emersed spikes, produces axillary submerged spikes which produce fruits under water. In the sandy, silt-covered bays of many lakes in the Adirondack Mountains, *P. spirillus* grows in association with such delicate aquatics as *Nitella tenuissima*, *Chara coronata*, and others, in water from one to three metres deep, where it may never come close enough to the surface to make wind pollination possible, but still it fruits in abundance and produces viable seeds. *Potamogeton obtusifolius*, frequently of shallow waters, has been found in Como Lake, Cayuga County, New York, fruiting in abundance in two metres of water under conditions where the water had not been lowered nor the inflorescences raised to extend above the surface of the water to permit wind pollination. *P. pusillus* frequently grows in deep water and fruits abundantly. On August 25th, 1935, this species was harvested in abundance, growing among *Nitella flexilis* in three metres of water in Hatch Lake, a small, clear, spring-fed lake with no inlet and almost no outlet, in Madison County, New York. The plants were fruiting freely and produced viable seeds. None of the plants were more than one metre long, and it was ascertained from local residents that the water-level at no time was low enough to permit these plants to reach the water surface. These illustrations indicate that at least some of the Pusilli and Hybridi produce viable seeds under conditions which preclude wind pollination. They do not indicate by what agency the pollen is dispersed. Further investigations of the possibility of pollen

dispersal by water or by aquatic animals, cleistogamous development of the flowers, or parthenogenetic development of embryos are necessary before this problem can be answered.

The remains of the old winter buds persisting at the base of many of



FIG. 11. *Potamogeton pusillus*. A, seedling showing primary root 20 days after seed germination;  $\times 2$ . B, seedling showing primary root and cotyledon beginning to shrivel, 32 days after germination;  $\times 2$ . C, seedling March 2, 1935, 60 days after seed germination; cotyledon and primary root gone, axis resembling a winter bud;  $\times 2$ . D, winter bud, as it appeared when taken from a pond on March 2, 1935;  $\times 2$ .

the Pusilli plants have been interpreted (5) as indicating that such plants could not be the products of seed germination. In cultures of *P. pusillus* it was noticed that the seedlings rather consistently and early lose their

seedling characteristics and appear like 'winter buds'. In Fig. 11, A, B, C, are shown three drawings of seedlings of *P. pusillus* 20, 32, and 60 days after seed germination. The cotyledon and primary root begin to shrivel about one month after seed germination. By the end of two months the seedling with its short thickened axis (Fig. 11, C), after the cotyledon and primary root have disappeared, appears very similar to a winter bud (Fig. 11, D, drawn from a bud taken from a pond on March 2nd). It is very doubtful whether mature plants of *P. pusillus*, developed from such modified seedling axes, could be distinguished from plants originating from 'winter buds'. It seems entirely possible that one reason why plants of the Pusilli so consistently have the remains of 'winter buds' at their bases is that the product of a germinated seed, early in life, may develop into a state which has not been recognized as different from true winter buds produced as a result of vegetative propagation. A similar, but less consistent, tendency was observed in *P. epihydrus*.

#### V. SUMMARY.

The results presented indicate that the apparent scarcity of viable seeds and seedlings in certain species of *Potamogeton* is not due to the inability of these species to produce viable seeds. It has been demonstrated that a number of species of this genus produce viable seeds capable of germinating without undergoing a long rest period, provided the seeds are not allowed to become dry.

Germination was obtained from all eighteen species of which undried, water-stored seeds were available. Seeds that had been air-dried for two months or longer gave almost no germination.

*P. angustifolius*, *P. confervoides*, *P. foliosus*, *P. obtusifolius*, and rarely *P. capillaceus*, *P. natans*, and *P. gramineus*, germinated within two to three months after harvest, when stored in water in the laboratory.

In most species the percentage of germination was increased by first storing the seeds for two to six months in water at 1–3° C., a treatment which in general corresponds to the exposure to cold water received by the seeds under natural conditions during winter. Good germination was obtained from seeds that had been stored in water at 1–3° C. for one year. However, some seeds of several species germinated in cold storage if left longer than six months before transfer to germinators.

The eighteen species of *Potamogeton* in which viable seeds were demonstrated represent 12 subsections, 1 in the subgenus *Coleogeton* and 11 in *Eupotamogeton*, including four species in the subsection Pusilli (*P. foliosus*, *P. obtusifolius*, *P. panormitanus*, and *P. pusillus*) which frequently grow under conditions unfavourable for wind pollination, and possess a highly developed method of vegetative reproduction by winter buds.

The demonstration that these species produce viable seeds in spite of the unfavourable conditions for wind pollination under which they frequently grow, supplemented by the field observations that certain species, *P. obtusifolius*, *P. pusillus*, and *P. spirillus*, sometimes produce viable seeds under water, under conditions where wind pollination is highly improbable, raises the question whether some members of the Pusilli and Hybridi may not produce seeds without depending upon wind for pollen dispersal.

Structures resembling 'winter buds' rather than seedling plants were observed early in the development of the product of the germinated seed of *P. pusillus*. A plant developing from such a seedling would probably be mistaken for one produced by vegetative reproduction by a winter bud.

The knowledge of the existence of viable seeds and of the conditions necessary for their germination opens another approach to the problem of possible hybrids among species of Pusilli and related subsections of *Potamogeton*.

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# Cuscuta as a Parasite on Pteridophytes.

BY

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(State University of Iowa.)

With two Figures in the Text.

RECENTLY Singh (4) reported experiments in which he grew *Cuscuta reflexa* Roxb. on a fern host, *Athyrium pectinatum* Wall. Of two dozen pteridophyte species experimented upon by him this fern alone was successfully parasitized by the dodder. Field studies by Singh show that no cryptogram or gymnosperm was attacked, while practically all angiosperms were readily parasitized. Literature cited by Singh listed only angiosperm host plants.

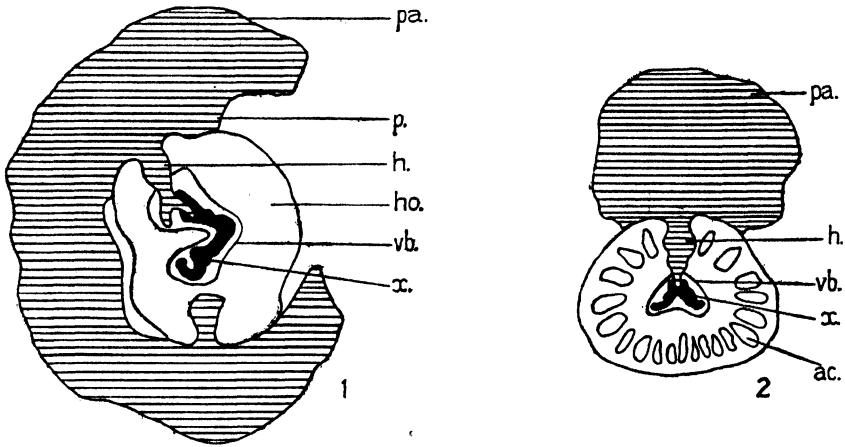
In 1859 Englemann (3) reported *Cuscuta* sp. growing 'even on the siliceous epidermis of Equisetum'. Thompson (5) in 1911 recorded *C. epithymum* Murr. as 'actually parasitical upon a couple of fronds of the limestone polypody (*Polypodium Robertianum* Hoff.)'. Yunker (6) in 1914 found *C. Gronovii* Willd. 'on one occasion coiling about and penetrating the stems of Equisetum'. Zender (8) in 1924 reported *C. epithymum* Murr. growing upon *Cystopteris fragilis* (L.) Bernh., and named *Dryopteris Filix-mas* Schott and *Asplenium Trichomanes* L. as host plants of *C. europaea* L. In addition, this same author described *Cuscuta* sp. penetrating the stem of *Equisetum variegatum* Schleicher. Later, Yunker (7) listed *Polypodium* sp. as a host plant for *C. Gronovii*.

In connexion with the writer's work (1, 2) on the host plants of *Cuscuta* 89 species were noted for *C. Gronovii* Willd. and 54 species for *C. glomerata* Choisy. One experimentally infested pteridophyte, *Marsilea Drummondii* A. Br., was included in the host list for *C. glomerata*. No pteridophyte host was then known to the writer for *C. Gronovii*. Later, however, two fern hosts, *Marsilea Drummondii* A. Br. and *Dryopteris dentata* (Forsk.) C. Chr., have been experimentally infested by the writer with this species of dodder.

In May, 1934, portions of an actively growing stem of *C. glomerata* were placed in contact with young petioles of *Marsilea* plants protruding above water in one of the greenhouses of the State University of Iowa. Within ten hours the dodder stem had coiled tightly about the host petioles, and prehaustoria were clearly visible the fourth day after attack. Haustoria had successfully penetrated the host tissues one week later, and

continued growth of the dodder readily established new coils of the parasite upon the same and adjacent *Marsilea* plants. This secondary growth is indisputable evidence of a successful primary attack.

The haustoria of the parasite penetrated the firm epidermis and loosely arranged cortical tissues of the host petiole and passed through the endo-



FIGS. 1 and 2. 1. Section through rachis of *Dryopteris dentata* showing branched haustorium within vascular bundle of host.  $\times 18$ . 2. Section through petiole of *Marsilea Drummondii* showing haustorium within vascular bundle of host.  $\times 20$ . (All tissues of the parasite in cross-hatching. *pa*, parasite; *h*, haustorium; *p*, prehaustorium; *x*, xylem; *vb*, vascular bundle; *ho*, host; *ac*, air chamber.)

dermis and pericycle to reach the xylem and phloem within. The haustoria were irregularly tapering, cylindrical organs (Fig. 2). The swollen shaft of the haustorium imbedded in the thick cortex of the petiole securely anchored this structure within the host tissues, and brush-like masses of haustorial-hyphae arising from its tip made connexion with the xylem and phloem of the vascular bundle. The fact of haustorial penetration and contact with the conductive elements of the host in conjunction with observed growth in length of the parasite stem indicated that attack of the dodder was in every respect successful. An abundance of starch grains in the stem and haustoria of the parasite further attested the success of this attack.

Not all such attempts were successful, however, since but one in ten of the experiments with this species resulted in contact of the haustoria with the vascular bundles of the host. The dodder stem failing to establish such contact soon withered and died. *Cuscuta Gronovii* attacked more vigorously than *C. glomerata* and more often parasitized the *Marsilea* hosts. Neither species of dodder grew well upon older, matured petioles.

Subsequently, successful infestations of another pteridophyte, *Dryopteris dentata*, were repeatedly brought about, using *C. Gronovii* as the attacking parasite. Specimens of this fern, growing in one of the greenhouses of the State University of Iowa, were attacked by dodder stems, unaided, from adjacent infested plants. This fern was also readily parasitized by detached

portions of vigorous *Cuscuta* stems experimentally twined round the stipe and rachis of the leaf. The haustoria of the parasite penetrated the epidermis and compact cortex, passing through endodermis and pericycle, and there dividing, one branch united with the xylem, the other made connexion with the phloem (Fig. 1). The swollen shaft and branched tip of the haustorium firmly anchored this organ in the host tissues. Similar forking was observed by Singh for the haustoria of *C. reflexa*. The younger shoots of *Dryopteris* were more readily parasitized than older, matured structures.

Once successfully established, the stem of the parasite grew rapidly in length, and increased greatly in thickness, leading to further attacks upon the same or adjacent *Dryopteris* plants. Iodine tests showed an abundance of starch in the stem and haustoria of the parasite at all stages. A further indication of successful attack was the bright orange-red colour of the dodder stems, since, with a deficient food supply, the stems of *Cuscuta* become a pale greenish yellow.

It is evident that some species of dodder are not wholly restricted to angiosperms, but may infest a variety of host plants. In localities where pteridophytes, especially delicate ferns, abound it is probable that infestation of these forms by *Cuscuta* would be of common occurrence.

#### SUMMARY.

1. Papers listing pteridophyte host plants of *Cuscuta* are reviewed.
2. Experimental methods used to infest two additional pteridophyte host plants are described.
3. The union of haustoria with bundles of the host plants is briefly discussed.

#### POSTSCRIPT.

Since this article was sent to press an additional reference (Gertz, O.: Till kännedomen om *Cuscuta europaeas* värdväxtflora. Bot. Not. 505-8, 1933) has been found in which *Dryopteris Filix-mas*, *Athyrium Filix-femina*, and *Equisetum pratense* are named as host plants of *Cuscuta europaea*.

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# The Interaction of Factors in the Growth of Lemna.

## IX. Further Observations on the Effect of Light Intensity on Growth and Multiplication.

BY

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(With Plate XX and five Figures in the Text.)

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### INTRODUCTION.

PAPERS in this series, which include observations on the effect of light intensity on the growth of Lemna, have been published by Ashby (1) Hicks (5), and Ashby and Oxley (3). Although there is general agreement [Annals of Botany, Vol. L. No. CC. October, 1936.]

between the work here presented and that of these authors, yet in detail there are notable differences, which justify further consideration of the effect of light intensity on growth. The differences are due to the following causes. (i) The effect of light intensity varies with strains of *Lemna minor* from different localities. This possibility has been noted by Ashby in an appendix to the paper by Hicks. Values are there presented of relative growth rates over a range of temperatures and light intensities (5, p. 524), which differ markedly from those obtained by Ashby and Oxley in later experiments (3, Table II). (ii) The effect of light intensity varies with the previous treatment of the colony and at different times, probably owing to seasonal changes in potentiality of growth, which are not eliminated by a controlled environment. The possibility of the effect of previous treatment and seasonal variation is referred to by Ashby and Oxley, who conclude that the 'occasion' on which the experiment is performed has no significant effect on the growth processes studied, so long as the colonies are kept under controlled conditions between each experiment. It is not clear if these authors maintain this position, for more recently (Ann. Bot., Corrigenda slip, L. no. cxcvii, 1936) they correct the 1 per cent. points in analyses of variance of their data which they gave earlier (3) and show that 'occasion', i.e. the effect of repetition of the experiments at two months' intervals, causes significant differences in frond weight. A similar correction, if applied to the 5 per cent. points (3, Table XI), would show that 'occasion' is also significant for frond area.

It would appear therefore that even with the same strain significant differences may be obtained at different times in spite of controlled experimental conditions. Evidence in support of this effect and of the response to light intensity of different strains at different seasons is presented in this paper.

#### EXPERIMENTAL PROCEDURE.

The experiments were carried out in an apparatus of similar type to that constructed by Ashby (1). This consists of four circular glass dishes 2 inches deep and 10 inches in diameter, each covered by a plate of glass and connected in series by glass tubes. Nutrient solution, made up of pure inorganic salts<sup>1</sup> with 'glass-distilled' water, is continuously circulated and aerated by the method devised by Ashby, Bolas, and Henderson (2). The apparatus holds 4.5 litres of solution which is replaced by fresh at 48-hour intervals, no variation in the pH value of 4.8 being noted during the experiments. The plant colonies are contained within square floating glass compartments, 2.0 inches across, each divided for convenience in counting

<sup>1</sup>  $\text{CaH}_4(\text{PO}_4)_2$  0.101 gm.;  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  0.581 gm.;  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  0.382 gm.;  $\text{MgSO}_4$  0.255 gm.;  $\text{K}_2\text{SO}_4$  0.668 gm.;  $\text{Fe}_2\text{Cl}_6$  0.002 gm.; dist. water 1,000 c.c.

into four smaller compartments. These 'floats' are anchored by means of a small glass tripod with prongs standing on the floor of each dish. The four dishes are placed under four gas-filled Mazda or Osram lamps, each lamp-holder being suspended by brass screw tubing, secured with nuts, from an arm of a wooden frame, mounted on a central stand. The lamps are enclosed in round-bottomed beakers of flowing cold water, secured by copper wire to enamelled lamp reflectors. The light intensity, estimated by a Holophane lumeter, is varied by employing lamps of different power and a finer adjustment obtained by raising and lowering the lamps by means of the screw tubing attached to the lamp holders. Each dish is painted on the outside with black varnish and light from above excluded by curtains of black silk fitted to the lamp reflectors. The four dishes are placed in a water bath maintained at constant temperature by carbon filament lamps, controlled by a thermo-regulator with contact between the platinum point and mercury in an atmosphere of hydrogen. All experiments were carried out at 25° C.

*Errors.* Since the method of experimentation precluded the use of duplicate colonies, the significance of the differences in mean relative rates of frond number increase is estimated from the daily fluctuations in growth, using the methods for the comparison of means introduced by Fisher (4). Relative rates of increase are represented by  $\frac{\log_e n_1 - \log_e n_0}{t_1 - t_0}$ , where  $n_1$  and  $n_0$  are the frond numbers at times  $t_1$  and  $t_0$  respectively, and daily counts provide a series of values from which the means and standard errors entered in the tables are calculated. From comparison of the variance of duplicate colonies in the experiments of Ashby and Oxley (3, Table III) with that which may be calculated from the daily fluctuations in multiplication rate of a representative colony (3, Table I) it appears that the extent of day-to-day differences exceeds appreciably that of differences between duplicate colonies, an effect which may be due to correlation between successive counts of frond number. It is probable therefore that differences in multiplication rate claimed in the present experiments are of greater significance than appears from the method of comparison used.

Samples were withdrawn every second day for the estimation of area by drawing an enlarged outline focussed on a sheet of ground glass and tracing with a planimeter, as described by Ashby, Bolas, and Henderson (2), and dry weight by drying in vacuo under standard conditions and weighing on a micro-chemical balance, as described by Su and Ashby (6). Allowance is made for the withdrawal of samples and the random removal of plants to prevent overcrowding when calculating the total number of fronds composing the colony. Errors of sampling have been published in a previous paper (7, Table I).

*Note on structure of the frond.* The morphological unit of growth in

Lemna is the 'frond'. In order to obtain an understanding of the structural characteristics of a frond, Lemna plants were fixed in Flemming's solution, embedded in paraffin wax, cut by microtome, stained with Haidenhain's iron-haemotoxylin and counter-stained with orange G. Illustrative sections are shown in Pl. XX, Figs. 1-4. Whereas the *morphological* character of a Lemna frond has remained a matter of dispute, its *physiological* character appears clearly to be that of a leaf, exhibiting the typical characteristics of an aquatic assimilating organ. Lacunae of large size are prominent, the assimilating tissue with chloroplasts occupies the upper portion of the frond and stomata, which appear to be non-functional, are confined to the upper surface.

TABLE I.

*Comparison of Relative Rates of Frond Multiplication of Two Lemna Strains from Different Localities.*

	$\bar{x} - \bar{y}$ .	$n$ .	$t$ .	$P$ .
300 foot-candles	0.069	10	1.58	0.15
600     "	0.056	10	2.26	0.05
900     "	0.048	10	1.18	0.27
1500    "	0.068	10	1.05	0.32

#### COMPARISON OF THE RESULTS OF EXPERIMENTS CARRIED OUT WITH DIFFERENT STRAINS AND AT DIFFERENT TIMES.

*Variation in strain.* The possibility of differences in relative growth rate under controlled conditions between strains of Lemna from different localities is suggested by Ashby (5). With the object of procuring a strain with growth response more varied than that already in use, colonies from several sources were tested from time to time. From the growth of colonies simultaneously in the diagonal squares of the same floating glass compartments, comparison is made in Table I between the mean differences in relative growth rate of strains from Kew and the Isle of Wight. In order to obtain an estimate of significance of the result *under all light intensities* advantage may be taken of the fact that the variance of the sum of a number of quantities is the sum of the separate variances. The sum of the mean differences divided by the standard error corresponding to the sum of the variances gives  $t = 2.62$  for  $n = 40$  and  $P < 0.01$ , thus demonstrating a differential response of strain to light intensity.

There appear to be a number of 'strains' of *L. minor*, apparently indistinguishable, but varying in their rates of growth under a given set of conditions. A more rapidly-growing strain may be considered preferable for experimental purposes, for it may be expected to respond more quickly to environmental changes.

*Differences in growth in experiments carried out at different times.*

During the summer of 1930 colonies of *L. minor* were grown under continuous light for a period of two months. The average frond dry weights are shown in Table II.

TABLE II.  
*Average Frond Dry Weight (mg.).*

	1200 ft.-candles.		900 ft.-candles.		600 ft.-candles.		300 ft.-candles.	
25. 5. 30	0.139 0.124	0.132	0.120 0.143	0.132	0.080 0.084	0.082	0.044 0.046	0.045
27. 5. 30	0.154 0.169	0.162	0.126 0.143	0.134	0.100 0.108	0.104	0.071 0.078	0.074
29. 5. 30	0.154	0.154	0.132 0.137	0.135	0.123 0.144	0.133	0.076 0.086	0.081
31. 5. 30	0.160 0.181	0.170	0.129 0.155	0.142	0.126 0.131	0.128	0.064 0.067	0.066
2. 6. 30	0.182	0.182	0.154	0.154	0.108	0.108	0.072	0.072
5. 6. 30	0.201 0.181	0.191	0.153 0.157	0.155	0.113 0.123	0.118	0.069 0.072	0.071
10. 6. 30	0.133	0.133	0.126	0.126	0.094	0.094	0.063	0.063
14. 6. 30	0.156 0.134	0.147	0.129 0.142	0.135	0.090 0.103	0.097	0.047 0.057	0.052
15. 6. 30	0.132 0.160	0.146	0.112 0.122	0.117	0.089 0.089	0.089	0.051 0.057	0.054
19. 6. 30	0.144 0.154	0.149	0.109 0.112	0.111	0.100 0.101	0.101	0.053 0.057	0.055
20. 6. 30	0.150 0.156	0.153	0.134 0.145	0.139	0.098 0.115	0.106	0.059 0.057	0.058
22. 6. 30	0.138 0.141	0.140	0.121 0.125	0.123	0.093 0.098	0.096	0.061 0.067	0.064
24. 6. 30	0.135 0.147	0.141	0.112 0.133	0.123	0.094 0.100	0.097	0.059 0.058	0.059
2. 7. 30	0.125 0.100	0.112	0.087 0.089	0.088	0.074 0.078	0.076	0.044 0.046	0.045
4. 7. 30	0.116 0.128	0.122	0.094 0.099	0.097	0.068 0.073	0.071	0.045 0.046	0.046
6. 7. 30	0.132 0.139	0.135	0.099 0.091	0.095	0.068 0.077	0.073	0.044 0.045	0.045
8. 7. 30	0.121 0.127	0.124	0.079 0.094	0.086	0.061 0.068	0.065	0.043 0.049	0.046
9. 7. 30	0.103 0.112	0.108	0.072 0.075	0.074	0.060 0.069	0.065	0.047 0.045	0.046
10. 7. 30	0.109 0.111	0.110	0.091 0.093	0.092	0.064 0.070	0.067	0.042 0.043	0.043
12. 7. 30	0.105 0.107	0.106	0.094 0.097	0.096	0.064 0.076	0.070	0.048 0.048	0.048
14. 7. 30	0.110 0.117	0.114	0.093 0.098	0.096	0.071 0.076	0.074	0.048 0.050	0.049

A steep preliminary rise, covering a period of adjustment from different conditions, is followed by a falling trend. This fall is roughly related to the

light intensity being steepest under 1,200 foot-candles, and least steep under 300 foot-candles. Since the standard deviations due to sampling, obtainable from the variance as between duplicates, are 3.5 per cent., 4.5 per cent., 4.4 per cent., and 5.2 per cent. for the light intensities from 300 to 1,200 foot-candles respectively, whereas the differences in frond weights between 5 June and 14 July are 31 per cent., 37 per cent., 38 per cent., and 40 per cent. respectively, the magnitude of the fall in dry weight is well beyond the limits of chance variation. This suggests that the view (p. 828) that the interaction of factors can be studied quantitatively by a series of successive experiments is not warranted.

The mean relative rates of increase in frond number  $\left( \frac{\log_e n_1 - \log_e n_0}{t_1 - t_0} \right)$  of strains from the Chelsea Physic Garden, the Isle of Wight, and Kew, at different periods of the year are shown in Table III. The standard errors attached are obtained from daily counts over a period of at least eight days, save in the first two experiments in which counts were made every second day.

Comparison is made, in Table IV, between the rates of frond number increase of the Chelsea strain under corresponding light intensities in April and May 1929. The differences are seen to be highly significant at each light intensity. The significance of the differences in rates of growth of the Isle of Wight strain in July and December is of a still higher order. Those of the Kew strain between November and January are significant at 900 and 1,200 foot-candles. Whereas the Isle of Wight strain obtained in 1932 may not be the same as that growing in the same pond in 1930, no such objection can be lodged against the strains from Chelsea and Kew, *which were grown continuously under the experimental conditions between the dates of observation*. It is clear that experiments under controlled conditions, if separated by intervals of time or carried out successively, but taking long periods to complete, do not necessarily yield similar results.

*Differential effect of light intensity upon growth in experiments carried out at different times.* Comparison of the relative rates of increase in frond number of Table III reveals that the differences between the same strain grown at different times are more marked under the higher light intensities. The type of response is illustrated in Text-fig. 1, contrasting the curves obtained from the Chelsea strain in May and July 1929. The percentage differences under corresponding light intensities are as follows :

Ft.-candles	300	600	900	1200	1500
Increment	1%	9%	12%	17%	22%

Text-fig. 1 suggests that, whereas the rates of growth at a low light intensity may be the same, the trend of curves under high light intensity is

TABLE III.

*Relative Rate of Increase in Frond Numbers (With S.E.).*

Origin of strain.	Light intensity in ft.-candles.	250	300	500	600
Chelsea	April 1929		$0.135 \pm 0.037$		$0.156 \pm 0.048$
Chelsea	May 1929		$0.223 \pm 0.013$		$0.255 \pm 0.012$
Chelsea	July 1929		$0.225 \pm 0.025$		$0.278 \pm 0.015$
Isle of Wight	July 1930		$0.235 \pm 0.008$		$0.292 \pm 0.013$
Isle of Wight	Nov. 1932	$0.201 \pm 0.044$		$0.215 \pm 0.014$	
Isle of Wight	Dec. 1932		$0.186 \pm 0.020$		
Kew	Nov. 1932	$0.187 \pm 0.024$		$0.220 \pm 0.021$	
Kew	Dec. 1932		$0.175 \pm 0.011$		
Kew	Jan. 1933		$0.157 \pm 0.011$		$0.192 \pm 0.020$

Origin of strain.	Light intensity in ft.-candles.	750	900	1000	1200	1500
Chelsea	April 1929		$0.157 \pm 0.043$		$0.166 \pm 0.036$	
Chelsea	May 1929		$0.270 \pm 0.022$		$0.271 \pm 0.026$	
Chelsea	July 1929		$0.302 \pm 0.019$			$0.331 \pm 0.039$
Isle of Wight	July 1930		$0.340 \pm 0.019$		$0.373 \pm 0.020$	
Isle of Wight	Nov. 1932	$0.242 \pm 0.024$		$0.247 \pm 0.019$		
Isle of Wight	Dec. 1932	$0.218 \pm 0.015$			$0.245 \pm 0.019$	
Kew	Nov. 1932	$0.279 \pm 0.015$		$0.295 \pm 0.007$		
Kew	Dec. 1932	$0.201 \pm 0.014$			$0.210 \pm 0.009$	
Kew	Jan. 1933		$0.206 \pm 0.017$		$0.208 \pm 0.007$	

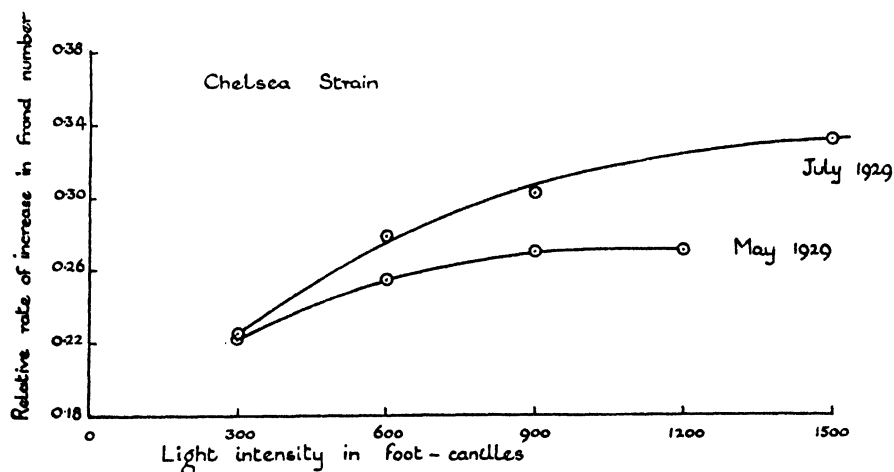
TABLE IV.

*Comparison of the Relative Rates of Frond Multiplication of a Single Strain in Successive Experiments under Controlled Conditions.*

	$\bar{x} - \bar{y}$ .	$t$ .	
300 foot-candles	0.088	5.00	$n = 7$
600 "	0.099	4.53	$P = 0.01$ for $t = 3.5$
900 "	0.103	4.55	
1200 "	0.105	4.85	



such that a significant difference might be observed. Such a case is provided by the growth of the Kew strain in November and January. The mean difference shown in Table V provides a value of  $t$  which is not significant at a low light intensity; but at a high light intensity the mean difference is more than nine times its standard error.



TEXT-FIG. 1. Relative rate of increase in frond number plotted against light intensity. The curves are drawn by eye through the experimental points.

TABLE V.

*Comparison by Fisher's Method of  $t$  of Relative Rates of Frond Multiplication of the Same Strain in Successive Experiments under Controlled Conditions.*

	$\bar{x} - \bar{y}$ .	$t$ .	$P$ .	$n = 14$
300 foot-candles	0.040	1.54	0.15	
600     "	0.057	1.97	0.07	
900     "	0.081	3.66	<0.01	
1200    "	0.094	9.39	<0.01	

*Discussion of differences in growth rate under controlled conditions at different times.* Quantitatively similar results have not been obtained in experiments carried out at different times, for the experimental results demonstrate the occurrence of differences of too great a magnitude to be attributed to chance. Change from different environmental conditions prior to the experiments does not account adequately for the results, for this possible source of error was eliminated between certain of the experiments by continuous growth of the colonies under the experimental conditions. It is of interest to note that the relative rates of frond number increase of the Chelsea strain rise from April to July and those of the Isle of Wight and Kew strains fall from July to December and November to January respectively. This suggests that the annual cycle of rate of

increase in frond number under natural conditions is not entirely eliminated by a constant environment so that the growth rates are at a maximum in late summer and at a minimum in late winter.

Variation in growth under controlled conditions at different times suggests the desirability of avoiding *quantitative* comparison between experiments carried out at different times. In view of the results set out in this paper it was decided, in further experiments on the mode of interaction of light intensity with the nitrogen and potassium contents of the nutrient solution, to carry out all treatments simultaneously.

#### THE EFFECT OF LIGHT INTENSITY ON GROWTH.

*Frond number.* All the curves of frond counts, from which the means recorded in Table III are derived, are of exponential type. Two curves representative of those obtained by plotting the exponential indices of Table III against light intensity are illustrated in Text-fig. 2. The change of rate of frond multiplication with light intensity in Text-fig. 2 follows a logarithmic decrement law; there is no indication of a relatively sharp break in the curves demanded by the theory of 'limiting factors'.

Considerable variation is apparent in Table III in the levels of relative growth rate. The relative rate of increase (Text-fig. 2) of the Isle of Wight strain in July 1930 under a light intensity of 300 foot-candles is significantly higher than the relative rate of increase of the Kew strain in January 1933 under a light intensity of 1,200 foot-candles ( $t = 2.48$  for  $n = 14$ ,  $P = 0.03$ ). Both curves tend to become linear as they approach 300 foot-candles, showing that with light intensities of this order the rate of increase in frond number is almost proportional to the illumination. In the lower curve of Text-fig. 2 increase in light intensity above 900 foot-candles causes no further increment in rate of growth; in the upper curve of Text fig. 2 the rate of increase in frond number is still rising between 1,200 and 1,500 foot-candles.

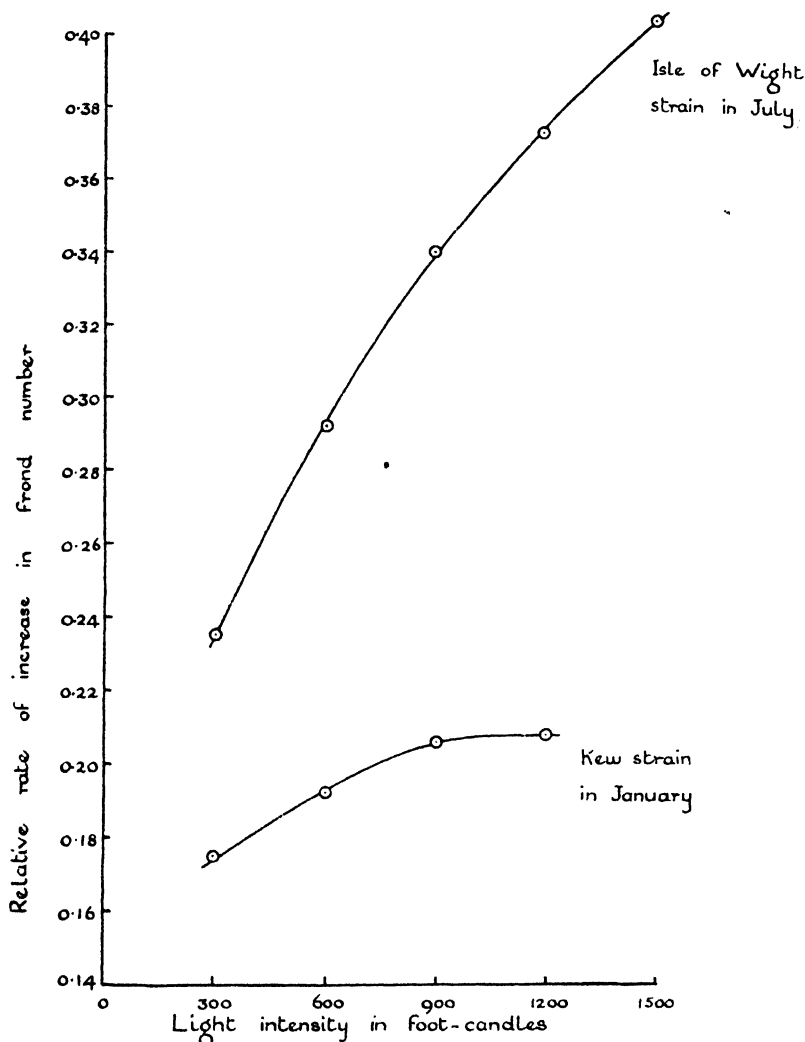
The effect on the Isle of Wight strain of increase in light intensity above 1,200 foot-candles was determined in an experiment immediately following that of July 1930, in which this strain was grown under light intensities of 1,500, 1,800 and 2,100 foot-candles. The results are shown in Table VI. There is no clear evidence of a fall with time in the daily indices of relative rate of increase in frond number, and the curves are treated as exponential. Table VII shows that the mean daily indices of frond multiplication under 1,800 and 2,100 foot-candles are lower than that under 1,500 foot-candles.

The frond areas under 1,800 and 2,100 foot-candles (Table VI) are clearly falling with time, in contrast to the areas of the 1,500 foot-candle colony, which rise with time, showing that the optimum has been exceeded and suggesting that growth could not be supported permanently under these light intensities. This is confirmed by the dry weight per unit area

TABLE VI.  
*Fronde Number, Area, and Dry Weight of Isle of Wight Strain under Supra-optimal Light Intensities.*

Foot-candles	1500	1800	2100	1500	Average frond area (sq. cm.)		2100	Dry weight per unit area (mg. per sq. cm.)			
					1800			1500	1800	2100	
18. 7. 30					0.0547 0.0576 0.0596 0.0590	0.0577			2.18 2.05 2.08 2.08		
19. 7. 30	100	100	100								
20. 7. 30	153	142	143	0.0513 0.0546	0.0521 0.0547	0.0534	0.0571 0.0565	2.60 2.67	2.46 2.27	2.37	1.89 2.00
21. 7. 30	216	201	195								
22. 7. 30	324	299	276	0.0583 0.0589	0.0571 0.0598	0.0585	0.0556 0.0564	2.64 2.71	2.26 2.31	2.29	2.05 2.09
23. 7. 30	509	439	394								
24. 7. 30	756	639	556	0.0593 0.0585	0.0589 0.0543	0.0566	0.0490 0.0516	2.45 2.52	2.38 2.43	2.41	.98 1.90
25. 7. 30	1120	970	819								
26. 7. 30	1690	1358	1141	0.0610 0.0603	0.0518 0.0560	0.0539	0.0524 0.0472	2.49 2.56	2.30 2.27	2.29	2.08 1.99
27. 7. 30	2590	2010	1650								
28. 7. 30	3810	2810	2340	0.0623 0.0684	0.0500 0.0539	0.0520	0.0479 0.0452	2.58 2.46	2.32 2.08	2.20	1.90 1.59
29. 7. 30	5760	4350	3220								

which is lower under 1,800 and 2,100 than under 1,500 foot-candles. The fall with time in frond area under 1,800 foot-candles was associated with a progressive paling of the frond colour, presumably associated with chlorophyll destruction. At 2,100 foot-candles the centre of each mature frond became



TEXT-FIG. 2. Relative rate of increase in frond number plotted against light intensity. The curves are drawn by eye through the experimental points.

characterized by a white bleached spot which enlarged as the experiment proceeded. Temperature measurements showed that this effect was due to the light intensity and not to radiant heat passing through the water-screens.

The optimal light intensity in the experiment represented by the upper curve of Text-fig. 2 lies between 1,500 and 1,800 foot-candles, in contrast to

the experiment represented by the lower curve of Text-fig. 2, in which the optimal light intensity does not exceed 900 foot-candles. The light optimum for multiplication of Lemna clearly has no fixed value, and varies with the

TABLE VII.

*Relative Rate of Increase in Frond Number of Isle of Wight Strain under Supra-optimal Light Intensity (with S.E.).*

1500 foot-candles	0.405 ± 0.009
1800       ,,	0.378 ± 0.011
2100       ,,	0.348 ± 0.008

TABLE VIII.

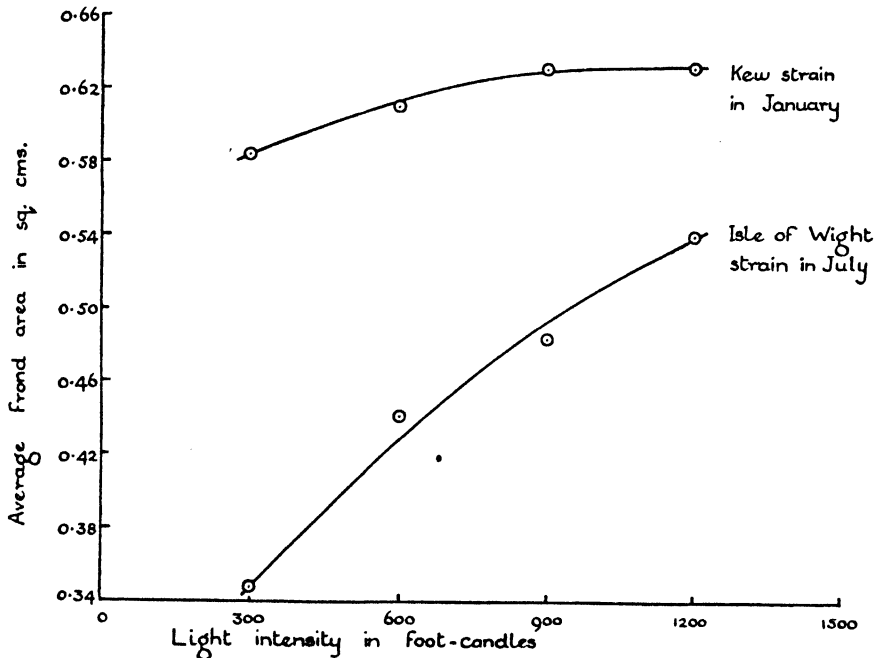
*Average Frond Area (sq. cm.).*

		Isle of Wight strain.							
		300 ft.-candles.		600 ft.-candles.		900 ft.-candles.		1200 ft.-candles.	
2. 7. 30		0.0351	0.0344	0.0460	0.0456	0.0482	0.0472	0.0459	0.0492
		0.0337		0.0451		0.0463		0.0525	
4. 7. 30		0.0344	0.0336	0.0418	0.0425	0.0526	0.0514	0.0550	0.0540
		0.0328		0.0432		0.0502		0.0529	
6. 7. 30		0.0349	0.0361	0.0482	0.0457	0.0513	0.0493	0.0574	0.0569
		0.0372		0.0431		0.0473		0.0563	
8. 7. 30		0.0335	0.0348	0.0404	0.0424	0.0434	0.0454	0.0542	0.0564
		0.0360		0.0443		0.0474		0.0585	
		Kew strain.							
		300 ft.-candles.		600 ft.-candles.		900 ft.-candles.		1200 ft.-candles.	
14. 1. 33		0.0569	0.0591	0.0659	0.0634	0.0609	0.0632	0.0654	0.0635
		0.0612		0.0609		0.0654		0.0616	
15. 1. 33		0.0569	0.0570	0.0631	0.0623	0.0645	0.0642	0.0706	0.0675
		0.0571		0.0614		0.0638		0.0654	
16. 1. 33		0.0653	0.0642	0.0623	0.0637	0.0637	0.0640	0.0664	0.0659
		0.0631		0.0650		0.0643		0.0654	
17. 1. 33		0.0621	0.0625	0.0644	0.0644	0.0662	0.0668	0.0587	0.0607
		0.0629		0.0643		0.0674		0.0626	
18. 1. 33		0.0549	0.0539	0.0592	0.0579	0.0631	0.0625	0.0671	0.0670
		0.0529		0.0566		0.0618		0.0668	
19. 1. 33		0.0559	0.0554	0.0609	0.0612	0.0646	0.0636	0.0591	0.0590
		0.0548		0.0615		0.0626		0.0589	
20. 1. 33		0.0550	0.0563	0.0592	0.0592	0.0616	0.0617	0.0614	0.0597
		0.0576		0.0592		0.0618		0.0579	
21. 1. 33		0.0602	0.0586	0.0571	0.0568	0.0592	0.0596	0.0632	0.0630
		0.0569		0.0565		0.0600		0.0628	

strain as suggested by Ashby (5). All the strains so far tested show, however, a light-growth relationship of a logarithmic decrement type.

*Frond area.* The average frond areas, corresponding to the rates of frond multiplication summarized in Fig. 2, are shown in Table VIII. The mean frond areas for the period of each experiment are shown in Table IX, and plotted against light intensity in Fig. 3. The curves of rate of change

of frond area with light intensity correspond in form to those of rate of change of frond multiplication, i.e. they follow a logarithmic decrement law. The relative levels of average frond area are, however, the reverse of those noted in Text-fig. 2 for rate of increase in frond number. The Isle of Wight



TEXT-FIG. 3. Average frond area plotted against light intensity. The curves are drawn by eye through the experimental points.

strain multiplies more rapidly, but has a smaller frond area, whereas the Kew strain multiplies more slowly, but has a larger frond area. The net effect is a tendency to make the increase in total area of the colony a constant. This trend has been noted in other strains, though data are insufficient to determine if it holds generally. It seems possible that differentiation of frond primordia has an adverse effect on growth of the parent fronds, so that earlier differentiation tends to reduce the average size of the parent fronds, leading to an inverse relationship between rate of frond multiplication and frond size.

The relative effects of light intensity on rate of increase in frond number and average frond area are shown in Table X. The increments in frond area of the Isle of Wight strain for a rise of 300 foot-candles correspond with those of rate of increase in frond multiplication. In contrast, the relative increase in frond area of the Kew strain is only 8 per cent. for a rise of 900 foot-candles, and it appears that the area of this strain is relatively unaffected by variation in light intensity.

*Net assimilation rate.* The net assimilation rates have been estimated by

$$\frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{A_2 - A_1}$$

where  $A_1$ ,  $A_2$ ,  $W_1$ ,  $W_2$  are the total areas and total dry weight of the colonies at times  $t_1$  and  $t_2$  respectively. The mean net assimilation rates

TABLE IX

*Average Frond Area (sq. cm.) with S.E.*

	Isle of Wight strain.	Kew strain.
300 foot-candles	0.0347 ± 0.0005	0.0584 ± 0.0012
600     "	0.0441 ± 0.0009	0.0611 ± 0.0010
900     "	0.0484 ± 0.0013	0.0632 ± 0.0007
1200    "	0.0541 ± 0.0028	0.0633 ± 0.0012

TABLE X.

*Relative Effect of Light Intensity on Frond Number and Frond Area.*

	Isle of Wight strain.		Kew strain.	
	Frond number.	Frond area.	Frond number.	Frond area.
300 foot-candles	0.235 100	0.0347 <sup>1</sup> 100	0.157 100	0.0584 100
600     "	0.292 124	0.0441 127	0.192 122	0.0611 105
900     "	0.340 149	0.0484 139	0.206 131	0.0632 108
1200    "	0.373 159	0.0541 156	0.208 132	0.0633 108
1500    "	0.405 172	0.0594 171		

TABLE XI.

*Net Assimilation Rate.*

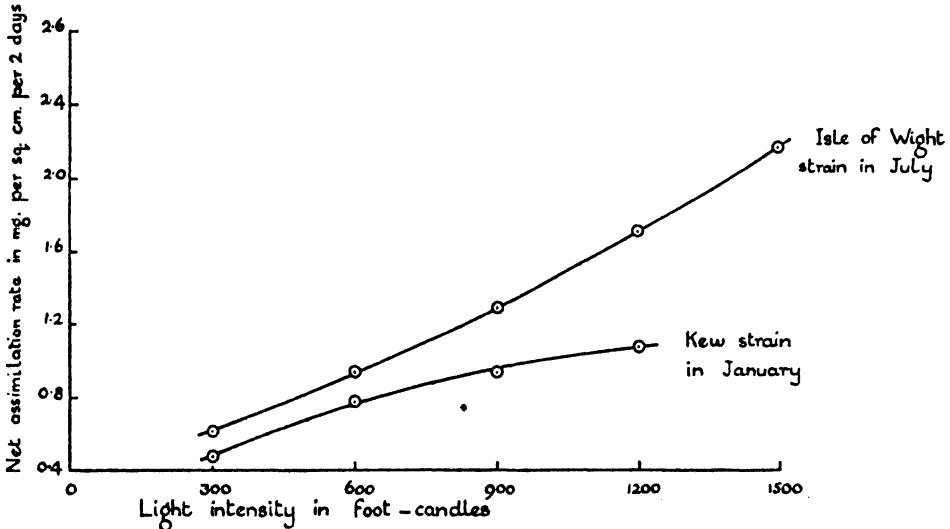
	Isle of Wight strain.		Kew strain.	
	Mg. per sq. cm. per 2-day period.	Mg. CO <sub>2</sub> per sq. dm. per hour. <sup>1</sup>	Mg. per sq. cm. per 2-day period.	Mg. CO <sub>2</sub> per sq. dm. per hour. <sup>1</sup>
300 foot-candles	0.62	2.1	0.47	1.6
600     "	0.94	3.2	0.78	2.7
900     "	1.29	4.4	0.94	3.2
1200    "	1.71	5.8	1.08	3.7
1500    "	2.18	7.4		

corresponding to the rates of frond multiplication and frond areas given in Text-figs. 2 and 3, are shown in Table XI and Text-fig. 4.

Fig. 4 shows that the successive increments in net assimilation rate per rise of 300 foot-candles of the Kew strain are falling, giving a curve concave to the light intensity axis, whereas those of the Isle of Wight strain appear to be rising slightly until 1,500 foot-candles is reached, giving a curve convex to the light intensity axis. At 1,800 foot-candles the net assimilation rate is lower than under 1,500 foot-candles, and Table XII shows that at this light intensity it is falling with time.

<sup>1</sup> The calculation of these values is based on the assumption that the composition of the dry frond material corresponds to the formula for starch or cellulose.

The termination of a rising curve of net assimilation rate with light intensity by the occurrence of values that fall with time indicates that with increasing light intensity a point is reached at which some component of the assimilation mechanism is destroyed. The occurrence of bleaching on exposure to yet higher light intensity (p. 837) supports the view of Ashby (1) that it is the chlorophyll that is primarily affected by supra-optimal light.



TEXT-FIG. 4. Net assimilation rate plotted against light intensity. The curves are drawn by eye through the experimental points.

TABLE XII.

*Net Assimilation Rate of Isle of Wight Strain under 1,800 Foot-Candles.*

	Mg. per sq. cm.
20-22. 7. 30	1.86
22-24. 7. 30	1.82
24-26. 7. 30	1.53
26-28. 7. 30	1.46

Two stages of the effect of increasing light intensity upon chlorophyll content must be distinguished. (1) A progressive reduction in chlorophyll content, demonstrated by Hicks (5), as the light intensity is increased above the relatively low light optimum for chlorophyll production of 300 foot-candles; the net assimilation rate is not appreciably affected. (2) Bleaching of the chlorophyll, setting in about 1,800 foot-candles, associated with a falling net assimilation rate.

The approximately direct relation between light intensity and net assimilation rate up to a light intensity at which the net assimilation rate



falls with time shows that (1) carbon dioxide has not limited assimilation under the experimental conditions, (2) the relative rate of increase in frond number is affected by a factor which is not assimilation rate, for the incre-

TABLE XIII.  
*Dry Weight per Unit Area (mg. per sq. cm.).*

Isle of Wight strain.									
		300		600		900		1200	
		ft.-candles.		ft.-candles.		ft.-candles.		ft.-candles.	
2. 7. 30		1.25		1.70		1.85		2.18	
	1.31	1.37		1.64	1.67	1.88	1.87	2.38	2.28
4. 7. 30		1.34		1.63		1.88		2.33	
	1.26	1.37		1.69	1.66	1.87	1.88	2.20	2.27
6. 7. 30		1.29		1.60		1.93		2.42	
	1.24	1.18		1.58	1.59	1.92	1.93	2.34	2.38
8. 7. 30		1.28		1.51		1.82		2.23	
	1.32	1.36		1.54	1.53	1.98	1.90	2.17	2.20
Kew strain.									
		300		600		900		1200	
		ft.-candles.		ft.-candles.		ft.-candles.		ft.-candles.	
14. 1. 33		1.44		1.90		2.45		2.57	
	1.47	1.50		2.28	2.09	2.13	2.29	2.60	2.59
15. 1. 33		1.53		2.06		2.16		2.71	
	1.57	1.61		2.15	2.11	2.20	2.18	2.62	2.67
16. 1. 33		1.47		2.10		2.18		2.59	
	1.47	1.46		2.15	2.13	2.13	2.16	2.72	2.66
17. 1. 33		1.45		1.97		2.18		2.38	
	1.46	1.46		1.99	1.98	2.26	2.22	2.46	2.42
18. 1. 33		1.44		1.92		2.25		2.64	
	1.45	1.45		2.00	1.96	2.25	2.25	2.72	2.68
19. 1. 33		1.45		1.90		2.34		2.60	
	1.48	1.50		1.92	1.91	2.28	2.31	2.58	2.59
20. 1. 33		1.43		2.01		2.42		2.41	
	1.47	1.51		2.01	2.01	2.43	2.43	2.58	2.50
21. 1. 33		1.49		1.95		2.38		2.60	
	1.55	1.60		1.93	1.94	2.40	2.39	2.68	2.64

ments in relative rate of increase in frond number begin to fall off at a light intensity not higher than 600 foot-candles in Text-fig. 2.

The increments in net assimilation rate for a rise of 300 foot-candles of the Kew strain fall with time, but a comparison of this curve (Text-fig. 4) with the corresponding curve of relative rate of increase in frond number (Text-fig. 2) demonstrates that the latter flattens out at 900 foot-candles, whereas the former is still rising at 1,200 foot-candles. The fall in net assimilation rate with rise of light intensity is thus insufficient to account for the relative falling away in rate of increase in frond number, again indicating the influence on multiplication rate of a factor other than assimilation rate.<sup>1</sup>

<sup>1</sup> C. F. Ashby and Oxley (8), who give curves relating net assimilation and the relative rate of frond multiplication, and conclude 'light intensity has some direct quantitative effect upon multiplication rate other than that acting through assimilation rate'.

*Dry weight per unit area.* Dry weight per unit area is given in Table XIII (for frond areas see Table VIII) and the mean dry weight per unit area under all light intensities in Table XIV (for frond areas see Table IX). The dry weight values are presented as mg. per sq. cm. rather

TABLE XIV.  
*Mean Dry Weight per Unit Area. (mg. per sq. cm.)*

	Isle of Wight strain.	Kew strain.
300 foot-candles	$1.31 \pm 0.02$	$1.49 \pm 0.02$
600     "	$1.61 \pm 0.03$	$2.01 \pm 0.03$
900     "	$1.90 \pm 0.01$	$2.28 \pm 0.03$
1200   "	$2.28 \pm 0.04$	$2.59 \pm 0.03$
1500   "	$2.57 \pm 0.04$	

than mg. per frond for the assimilation of a colony, and should be related to the area exposed to illumination rather than to the rate of frond multiplication. It is of interest to note that the errors of estimation of dry weight per unit area between duplicate samples are consistently less than those of frond weight or frond area of the same samples.<sup>1</sup> This effect must be due to an inverse correlation between frond area and frond dry weight, fronds that are below the mean level of the colony in area tending to have values above the mean level of the colony in dry weight.

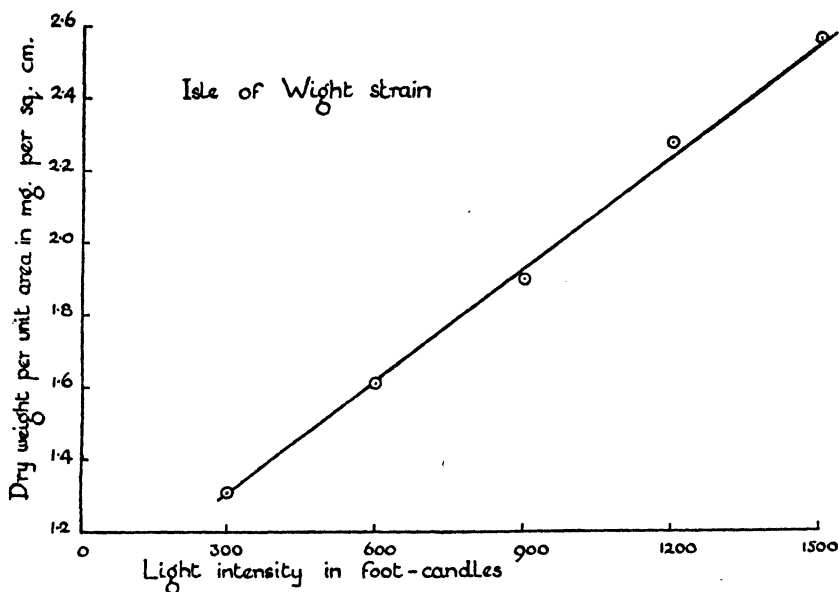
Fig. 5 shows that the dry weight per unit area of the Isle of Wight strain is directly proportional to the light intensity up to the value where the net assimilation rate falls with time. In contrast the increments in dry weight per unit area of the Kew strain (Table XIV) fall with increase in light intensity in accordance with the law of logarithmic decrement.

*Amylolytic activity.* In order to compare the effect of variation in light intensity on the rate of starch hydrolysis, samples calculated to be of equivalent dry weight were taken from colonies of the Isle of Wight strain grown under 300, 750, and 1,200 foot-candles. After grinding with sand the same volume of a standard starch solution was added to each sample, which was shaken and incubated for 40 minutes at 50°C. After dilution one drop of iodine solution was added to each tube. It was found that all the starch had been hydrolysed by fronds from the colony grown under 300 foot-candles, and it was estimated, by colour comparison with control tubes, that two-thirds and one-third of the starch had been hydrolysed by fronds from the colonies grown under 750 and 1,200 foot-candles respectively. The rates of starch hydrolysis on a dry weight basis thus fall off with increasing light intensity. The average frond areas of these colonies in sq. cm. were 0.0448 at 300 foot-candles, 0.0472 at 750 foot-candles, and 0.495 at 1,200 foot-candles;

<sup>1</sup> The standard deviation appropriate to the duplicate samples of the Isle of Wight strain in Table XIII is 2.8 per cent., whereas that of frond dry weight is 5.1 per cent. and frond area is 4.1 per cent. (see also 7, Table I).

on an area basis also high rates of starch hydrolysis must have been associated with low light intensity.

*Chlorophyll and protein content.* In these experiments the fronds of deepest green colour denoting highest chlorophyll content were formed at



TEXT-FIG. 5. Dry weight per unit area plotted against light intensity. The curve is drawn by eye through the experimental points.

a light intensity of 300 foot-candles. The fronds of maximal protein content, shown by decoloration in alcohol and staining with Millon's reagent, were also formed at a light intensity of 300 foot-candles. From other experiments<sup>1</sup> it is known that both above and below this light intensity the chlorophyll and protein contents fall off.

#### DISCUSSION OF THE EFFECT OF LIGHT INTENSITY ON GROWTH.

Attention may be directed to several interesting features of the effect of light intensity on the growth of *Lemna* colonies.

*The occurrence of physiological strains.* The different physiological behaviour of strains of *Lemna* from different localities emphasizes the necessity for caution in the interpretation of the relationships between light intensity and the growth processes of *Lemna* colonies.

*The quantitative effect of increasing light intensity on frond multiplication.* The smoothness of the curves of Text-fig. 2 drawn through the experi-

<sup>1</sup> The behaviour of *Lemna* colonies as the light-intensity is reduced below 300 foot-candles will be considered in detail in later papers of this series.

mental values leaves no doubt that the relationship between relative rate of increase of a *Lemna* colony and light intensity is of logarithmic decrement type. A progressive falling away from proportionality with increase of a single factor may be considered as due either to the increasing effect of a relative deficiency of some other factor or factors, or to a direct inhibiting effect of the increase of that factor. In the present case the concentrations of the more important nutrient salts appear to have been adequate for the highest rate of increase recorded (7), (8); carbon dioxide was not limiting (p. 842); the temperature was approximately optimal (3) and it seems improbable that other environmental factors were of sufficient importance to have had an effect of the magnitude shown. The relative falling away in multiplication rate is attributed rather to a progressive inhibiting effect of increasing light intensity. This detrimental influence is clearly independent of the photosynthetic mechanism, as shown by the direct relation between dry weight and light intensity (Text-figs. 4 and 5). It may be significant that the falling off in the increments of multiplication rate runs parallel with decreasing chlorophyll and protein content (p. 844). Nitrogen is known to be of prime importance for meristematic activity, and it seems probable that a fall in protein content would have a retarding effect on the development of frond primordia, leading to a relative fall in the increments of multiplication rate with increase in light intensity. These considerations suggest that the factor that affects multiplication rate independently of assimilation (p. 842 and footnote quoted on p. 842) may well be the decreasing protein content that characterizes colonies grown under increasing light intensity.

*The level of optimal light intensity.* The value of the optimal light intensity for the growth of *Lemna* colonies is a variable characteristic depending on the strain and probably other factors such as the previous environment. The values of the light optima for increase in frond number recorded by Ashby (1), Hicks (5), Ashby and Oxley (3), and the author lie between 750 and 1,800 foot-candles for *continuous* illumination.

The view that depression of growth processes at high light intensity is due primarily to a harmful effect of light on the chlorophyll mechanism is held by Ashby (1). It is of interest to note that the detrimental effect of supra-optimal light intensity in the present experiments is more pronounced on the net assimilation rate (see p. 841) and frond area (Table VI) than on the rate of frond multiplication (p. 835). The relatively low light intensities of 1,800 foot-candles (p. 837) and 1,400 foot-candles (1), at which destruction of the chlorophyll of *Lemna* has been observed, contrast with the 8,000 foot-candles to which vegetation is exposed on a clear summer's day. *Lemna* under these experimental conditions is exposed to *continuous* illumination; under natural conditions it seems possible that any loss of chlorophyll during a few hours of bright sunshine would be restored during the following period of darkness. Ashby's results (1) are in agreement with this supposition, for

he shows that the detrimental effect of high light intensity on rate of increase in frond number is reduced by a period of darkness.

*The lack of response in frond area to variation in light intensity.* Hicks (5) and Ashby and Oxley (3) are unable to detect any effect of light intensity on frond area and conclude that this measure of growth is unaffected by light intensities above 350 foot-candles. In the writer's experiments increase in light intensity has a positive effect on frond area, but the response is not always pronounced; and in an experiment with a strain from the same locality as that used by Ashby and Oxley there is an increase of only 8 per cent. corresponding to a rise in light intensity from 300 to 1,200 foot-candles. In most experiments the effect on frond area of increase of light intensity above 300 foot-candles is slight in comparison with the corresponding effect on rate of frond multiplication and increase in dry weight.

By decolorizing fronds in alcohol and staining with iodine it is readily seen that the starch content increases as the light intensity is increased above 300 foot-candles. The protein content, however, as shown by the staining of decolorized colonies with Millon's reagent, decreases as the light intensity is increased and is greatest at a light intensity of 300 foot-candles. Moreover, the amylolytic activity increases as the light intensity is reduced from 1,200 to 300 foot-candles (p. 843), a fact which is in conformity with the hypothesis advanced in a previous communication (8) of a relation between protein content and the rate of starch hydrolysis. These considerations suggest that a relatively greater proportion of the carbohydrate formed is present as starch at high light intensity and as sugar at low light intensity. Evidence has been submitted previously (8) that the frond area is affected by the sugar content and evidence will be presented in the later papers of this series of a correlation between frond area and protein content. As the light intensity is increased from 300 to 1,200 foot-candles the assimilation rate is increasing and this would increase the frond area. On the other hand, the protein content falls with increasing light intensity and there appears to be a tendency for a greater proportion of the photosynthate formed to be present as starch and cellulose instead of as sugar; these effects would reduce the frond area. The net result would be a tendency for the average frond area to remain relatively unaffected by variation in light intensity.

*The potentiality of dry weight increase under optimal light intensity.* The colony of the Isle of Wight strain under 1,500 foot-candles is of special interest as its daily exponential index of growth,  $0.405 \pm 0.009$ , measured over a period of ten days of continuous illumination, is believed to be the highest rate of growth increase yet recorded in a higher plant.<sup>1</sup>

<sup>1</sup> A single plant of *Lemna* of four fronds of the same average area and weight as in this experiment, continuing to increase at this rate for a period of three months, would form a colony of  $238 \times 10^{18}$  fronds with a weight of 600,000 tons and covering an area of 6,000 square miles. Such a dry weight could only be attained by the fixation of nearly a million tons of carbon dioxide.

## SUMMARY.

1. Strains of *L. minor* from different localities, apparently indistinguishable, show different relative growth rates under constant conditions.

2. Differences have been found in the growth rates of a single strain under controlled conditions in experiments repeated at intervals of time as short as two months. These differences tend to be slight at low light intensities, but significant at high light intensities. There is evidence that colonies grow more rapidly in summer than in winter, but the experiments were not sufficiently extensive to determine whether all the differences observed are to be explained by a natural annual cycle not entirely eliminated by the constant external conditions.

3. *The effect of light intensity. Frond number.* The curves of relative rate of increase in frond number plotted against light intensity indicate a direct relationship between rate of frond multiplication and light of low intensity. As the light intensity is increased the increments in rate of multiplication fall off progressively in accordance with the law of logarithmic decrement. For a strain from one locality the optimal light intensity lay between 1,500 and 1,800 foot-candles, but for a strain from a different locality the optimal light intensity was of a lower order, for increase in light intensity from 900 to 1,200 foot-candles did not increase the rate of frond multiplication.

*Frond area.* The effect on frond area of increase in light intensity above 300 foot-candles was appreciable in an experiment with a strain from one locality, but in an experiment with a strain from another locality the increase was slight relative to that of frond multiplication and dry weight. The nature of the slight response of frond area to variation in light intensity is discussed.

*Net assimilation rate.* For a strain from one locality the curve relating light intensity and net assimilation rate was convex to the light intensity axis between 300 and 1,500 foot-candles. At 1,800 foot-candles the net assimilation rate fell with time. With a strain from a different locality a curve concave to the light intensity axis was obtained.

*Dry weight per unit area.* The dry weight per unit area of a strain from one locality was directly related to the light intensity between 300 and 1500 foot-candles. With a strain from a different locality the curve relating dry weight per unit area and light intensity was of a logarithmic decrement type.

*Chlorophyll and protein content and amylolytic activity.* Under the experimental conditions (continuous illumination at a temperature of 25° C.) fronds of maximal chlorophyll and protein content are formed at the relatively low light intensity of 300 foot-candles. As the light intensity increases there is a relative falling off in multiplication rate, which is not

completely controlled by the assimilation rate but is associated with a falling protein content. The chlorophyll content also falls at light intensities sub-optimal for multiplication rate but without a detrimental effect on net assimilation rate. Further increase in light intensity leads to destruction of the chlorophyll and a fall in net assimilation rate. The capacity of extracts of *Lemna* fronds for hydrolysing starch decreases as the light intensity is increased above 300 foot-candles.

4. A rate of dry weight increase of over 40 per cent. compound interest per day is recorded, which is believed to be the maximum rate yet observed in a higher plant.

Grateful acknowledgements are due to Professor V. H. Blackman for the provision of facilities for carrying out the work and to Dr. E. Ashby for introduction to the experimental technique, which was essentially the same as that used by him in earlier experiments.

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#### EXPLANATION OF PLATE XX.

Illustrating Dr. H. L. White's paper on 'The Interaction of Factors in the Growth of *Lemna*'. IX.

Fig. 1. Section of a frond showing prominent air-spaces, assimilating tissue with chloroplasts below a suberized epidermal layer and stomata confined to the upper surface.

Fig. 2. Section of a mature *Lemna* plant. Three generations of fronds have been cut, the youngest in a depression beneath the parent frond and within the shelter of a flap from a still older generation.

Fig. 3. Section through a stoma showing guard-cells. The appearance suggests that the stoma is non-functional.

Fig. 4. Section through a young meristem. The central cushion of young cells, each filled by a relatively large nucleus, is surrounded by a ring of dense granular cells, also with large nuclei. The outer protecting layers are loosely arranged (see also Fig. 2). The early-formed air-spaces, appearing as small triangular light areas around the central meristematic cushion, may be noted.







# On the Development of the Perithecium of *Microeurotium albidum*.

BY

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With twenty-six Figures in the Text.

THE fungus which is the subject of this investigation appeared as a contamination in cultures of the spores of *Poronia punctata*, and since this latter form is coprophilous it is probable that *Microeurotium albidum* has a similar mode of life.

## METHODS OF CULTURE.

The mycelium grows on a variety of stock media and fruits abundantly. Coon's medium proved the most satisfactory owing to its relative transparency, which renders it favourable for the direct observation of living material. Though the mycelium develops readily at room temperatures, the most favourable range is from 20° C. to 25° C.

For the study of the germination of the spores, hanging drops in van Tieghem cells were employed and the development of the mycelium and its reproductive structures observed on slide cultures. These were prepared by pouring an agar medium on 3 × 1 in. slides which, after infection, were placed in Petri dishes lined with wet blotting paper. These could at any time be flooded with sterile water, protected with a large cover-glass, and examined with medium powers on the stage of the microscope. This permitted the examination of the mycelium in situ and the whole course of its development to be followed on the living material.

*Fixation and staining.* For the study of the morphology of the archicarp and young perithecia, portions of the material were removed, fixed, and stained in bulk. Various dyes were found suitable for this purpose, picro-nigrosine and gentian violet staining both walls and contents, and acid fuchsin and cotton blue staining the protoplasm only. After staining, the material was cleared in glycerine and mounted in glycerine jelly.

The material was fixed in strong Flemming's solution diluted with an equal volume of water. Small blocks of agar were cut out of the culture

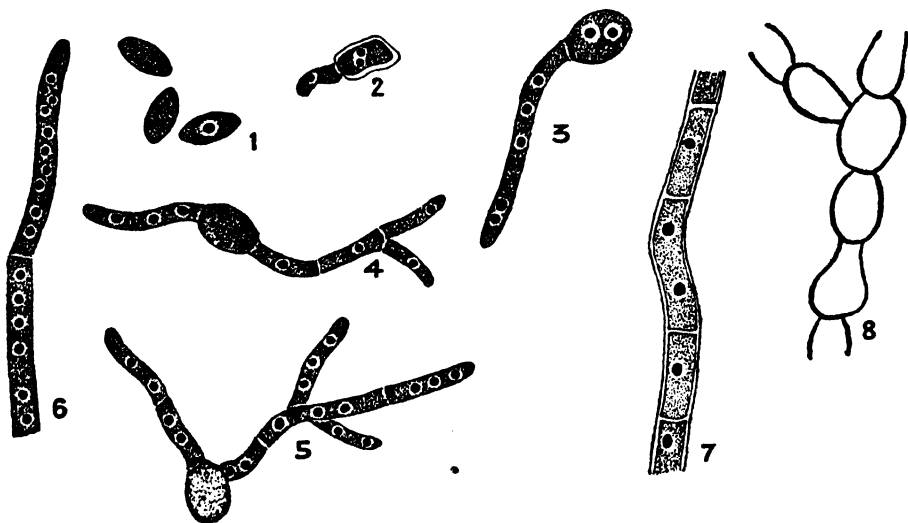
and immersed in the fixative for twenty-four hours. After washing, material for examination in bulk was bleached with hydrogen peroxide and transferred to the stain. For more detailed examination the material was dehydrated and embedded in the usual way. Sections were cut from  $6\mu$  to  $10\mu$  and stained with Heidenhain's haematoxylin—counter-stained with orange G in clove oil. Gentian violet proved useful when it was desirable to stain the cell-walls, slides being immersed for twenty-four and even forty-eight hours in a 2 per cent. solution, rinsed with water, quickly dehydrated and differentiated with orange G in clove oil.

*Spores.* The spores are elliptical in form, colourless and hyaline (Fig. 1). The size varied from  $5\mu$  to  $10\mu$  in length and  $3.5\mu$  to  $4.5\mu$  in width. The average size may be put as  $7.8\mu \times 4.1\mu$ . At the time of germination they take up water and increase in size. They are uninucleate in the resting condition, but may contain more than one nucleus soon after germination commences. This takes place seven or eight hours after inoculation, at temperatures varying from  $20^{\circ}\text{C.}$  to  $25^{\circ}\text{C.}$  The germ tubes, which may be one or more in number, generally make their way through the narrow ends of the spores, but cases are not rare where they emerge from the middle. They may come out from two opposite ends or all from one side (Figs. 4-5). During the course of their development they are at first unsegmented, but soon their rapid growth is followed by the formation of transverse septa and finally numerous branches. Thus within a short time a rapidly growing mycelium is formed. The germ tubes and each of their segments formed by septation contain several nuclei (Figs. 3-5).

*Mycelium.* The general appearance of the mycelial growth of this fungus is of special interest. The main branches radiate from the centre towards the circumference on which the lateral branches are formed in acropetal succession. So the whole culture assumes a circular form, and the branches show a dendroid appearance; a similar character was observed by Ramlow (12) in *Thelobolus Zukalii*. The surface of the culture forms a tough shiny coating, which is generally yellowish in colour. The width of the hyphae varied from  $1.5\mu$  to  $4\mu$  in different regions of the culture. Near the apical growing region, the hyphae have long segments with abundant protoplasm completely filling the cell cavity and very thin walls, hardly distinguishable from the cell contents. Passing towards the older region the segments gradually become shorter in length, the walls are appreciably thickened, and vacuoles appear in the protoplasm.

Quite a considerable number of nuclei are found in growing regions of the hyphae and can often be seen in division (Fig. 6). The transverse septa appear farther back, and each resulting segment contains several nuclei. Towards the older part of the mycelium the number is gradually reduced, till the cells become uninucleate. But, though this is the general rule, there are plenty of instances where, even in the very old parts a

binucleate condition prevails in cells intercalated between uninucleate ones (Fig. 7). The nuclei are exceedingly small in size and show no apparent differentiation in structure beyond a minute black nucleolus surrounded by a clear zone. Besides the normal type of hypha there is another kind



FIGS. 1-8. Fig. 1. Spores. Fig. 2. Germinating spore. Fig. 3. Germ tube, with several nuclei, still unsegmented. Figs. 4 and 5. Branching and segmentation of germ tubes. Fig. 6. Hyphal tip with multinucleate segments. Fig. 7. Older hypha with uninucleate and binucleate segments. Fig. 8. Hypha from old culture.

which differs both in form and in structure. There is a marked tendency for the older hyphae to swell in various points. This becomes very prominent in old cultures where considerable swelling takes place, and the cells assume an oval or spherical form (Fig. 8). The walls become extremely thin, and show very scanty contents. The size varies to a considerable extent, sometimes they are as large as the perithecia with which they are associated in large numbers in old cultures. Their positions in the hyphae vary in different cases, sometimes intercalary and occasionally terminal. They have not been observed to germinate and are therefore apparently not concerned with the reproduction of the fungus. It may be recalled that abnormal mycelial growths have been recorded as a familiar feature of old cultures of many different fungi. A striking feature of this fungus is the entire absence of conidia or other accessory reproductive bodies.

*Perithecium.* These spherical bodies are produced in large numbers within three to four days after inoculation. Every one of them arises from a single archicarp, the development of which was studied from its inception up to the maturity of the fruit body to which ultimately it gave origin.

The first appearance of the archicarp initial is a slight swelling on the lateral wall of an aerial hypha, at some distance from the growing apex.

In an hour's time the swollen portion bulges out and often assumes a club-shaped form, having its apical portion broad and rounded, the base slightly narrow (Fig. 9). Development takes place very rapidly. The branch elongates and becomes curved in about three hours' time (Fig. 10). Curvature takes place in every case, but its mode may vary in different forms. In the simplest type where the archicarp is very short, it bends slightly to form an arch. The next type is formed when the apex takes a little turn and comes quite close to the base, looking like a circle (Fig. 12). Here the whole body of the archicarp lies closely adpressed against the mother hypha. In such forms it is often very difficult to distinguish the base and apex, especially when the terminal portion does not become appreciably swollen. In other cases where the archicarps are fairly long different types of bending and twisting appear. The most common form is the bending of the apical portion like a hook towards the base of the stalk which stands erect on the mother hypha (Fig. 13), but in other cases they coil spirally or twist themselves in different ways.

Along with the development of the archicarp a new structure originates within five to six hours from its basal region (Fig. 12). This is a very thin and delicate branch, which gradually elongates and coils round the archicarp.

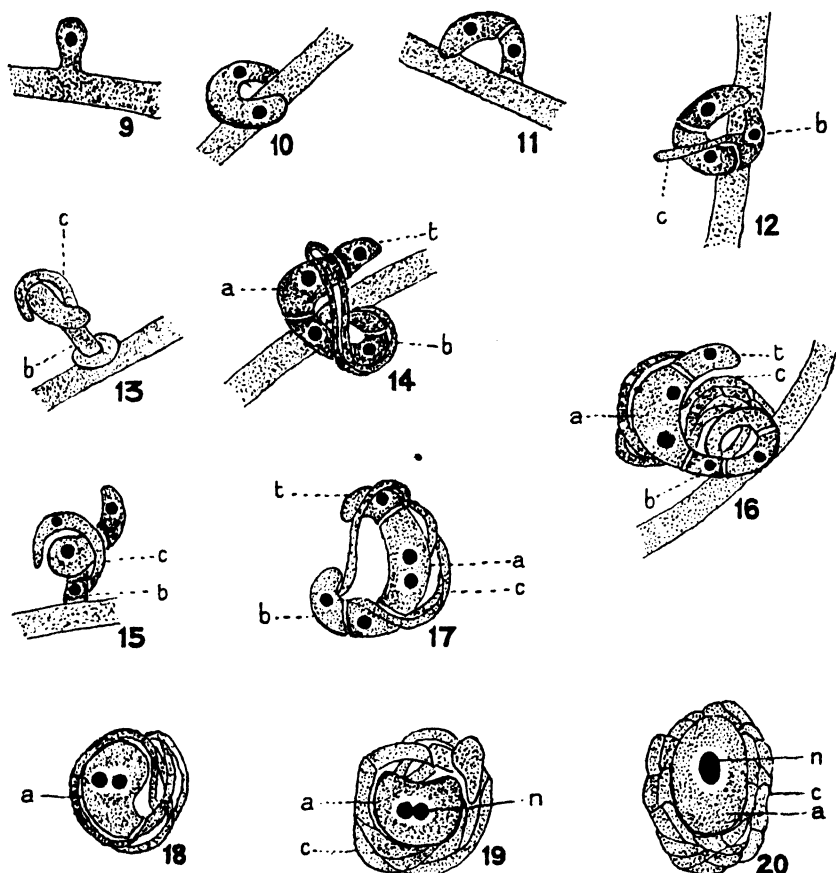
After three to four hours, from the first appearance of this filament at its base, the free end of the archicarp begins to swell, and this continues until it attains a spherical form. This body, the young ascus, grows further, and within about twenty-four hours becomes covered by loosely interwoven hyphae. Owing to the thinness and transparency of this covering sheath it is possible to study the structure of the enclosed ascus, while the development of the perithecium is in progress. The cytoplasm within appears very granular and divides into several portions. Gradually these take definite shape and develop into spores. A variable number of them is found embedded in the cytoplasm. The time required for the development of the ascus from the first appearance of the archicarp up to the formation of the spores is approximately three days.

As regards the covering filaments, their origin and further development could not be observed in detail in the living condition, except that they clearly have a mixed origin, some arising from the basal part of the archicarp and some from the vegetative hyphae.

The observations so far recorded were made on living material in which neither the walls nor the contents of the archicarp cells could be readily distinguished. The following observations are derived from the examination of fixed and stained material both entire and in sections. The young archicarp contains a single nucleus (Fig. 9) as a result of its migration from the mother hypha. A wall is then formed at the base so that the archicarp is composed of one cell, with a single nucleus. As growth continues, followed by curvature, the nucleus divides (Fig. 1c). The

resulting nuclei remain close together for some time and then move apart, one remaining at the base and the other migrating towards the apex (Fig. 10).

Meanwhile the entire archicarp assumes a curved form and a transverse



FIGS. 9-20. Fig. 9. Archicarp initial. Fig. 10. The original nucleus has divided into two. Fig. 11. Appearance of first septum. Fig. 12. Young archicarp divided into stalk, ascogonium and trichogyne, the first covering hypha (the antheridium?) arising from the stalk-cell. Fig. 13. Crozier-like archicarp with first covering hypha. Fig. 14. Archicarp with three-celled stalk. Fig. 15. The ascogonium beginning to enlarge. Figs. 16, 17. Binucleate ascogonium, the nuclei in the trichogyne and in the subtending stalk are shown; covering filaments growing up around the archicarp. Figs. 18-20. The ascogonial cell enlarging to form the young ascus and the nuclei fusing to form the definitive nucleus.

septum appears, separating it into cells, each containing one daughter nucleus (Fig. 11). The basal cell gives origin to the stalk, and the terminal one forms the ascogonium and the trichogyne of the fully-formed archicarp.

The lower cell undergoes further subdivision, the resulting cells, which may be two, three, or occasionally four, form the stalk of the archicarp.

The variation in number of stalk cells has been observed in specimens of the same culture grown under similar conditions.

While the stalk is being formed from the lower segment, the nucleus of the upper cell undergoes division producing two daughter nuclei. The cell elongates further, and one of these nuclei travels towards the apex. A transverse wall immediately appears to separate the cell into two, each containing one of those newly formed nuclei.

Of the two cells thus formed, the terminal one (Fig. 14) resembles in position the trichogyne of other ascomycetes, but its function in this case is uncertain. It does not behave as an organ for receiving and subsequently transferring a male nucleus to the ascogonial cell. The separating wall between it and the ascogonium persists to the last, so that there is no possibility either of cell fusion or of nuclear migration. The nucleus persists for some time, but ultimately degenerates with its surrounding cytoplasm. In later stages the cell may be found devoid of contents, and finally the whole structure disintegrates.

The middle cell, the ascogonium, which is intercalated between the stalk and the trichogyne, is from the first conspicuous by its greater length (Figs. 12–14). It has very thin walls, hardly distinguishable from the cytoplasm within, but entirely separating the contents on both sides from those of the trichogyne and the stalk cell. The cavity is completely filled with cytoplasm, which is very dense in consistency, and contains a single nucleus. As soon as it is cut off from the trichogyne it undergoes specialization both in size and form. Its bulk increases appreciably, and it gradually assumes a crescentic form. The nucleus increases in size, and shows a deeply-stained body, the nucleolus, in the centre, surrounded by a clear zone. This central body is very small and homogeneously stained, and does not show any structural differentiation.

The further development of the archicarp is not dependent upon fertilization by an antheridium. As already described, at the time when the archicarp undergoes segmentation a branch, which was at first identified as an antheridium, originates from the basal cell of the stalk (Figs. 12–14). It is very delicate and multicellular, completely filled with dense cytoplasm. Its general appearance, the place of origin, and the way it coils round the archicarp, may lead one to identify it as an antheridium similar to those described in *Aspergillus* (4, 9), but does not function as such. Although it coils round the archicarp, it does not reach the level of the trichogyne. Repeated search failed to reveal any direct connexion between its cell contents and those either of the trichogyne or of the subterminal cell of the archicarp, the ascogonium; careful search was made, with materials of different stages fixed at different times, but no indication was found of such fusion and consequently there was no free passage to allow migration of nuclei, as has been observed in other ascomycetes, e.g. *Pyronema* (10), where both

antheridium and archicarp grow together side by side. Considerations of the above-mentioned facts indicate that this hypha is not a functional male organ. On the other hand, it eventually contributes to the formation of the wall of the perithecium.

Of all the structures hitherto described, the subterminal cell of the archicarp plays the most important role in the subsequent development of the perithecium. As already described, this cell, the ascogonium, is responsible for giving rise to a single ascus. When first separated from the trichogyne, it is uninucleate, but this condition does not continue long. By the time the first covering filaments have developed two nuclei are to be seen embedded in the cytoplasm (Fig. 16). The cell attains this binucleate stage so quickly that it is extremely difficult to trace the origin of this condition. It has been shown above that there is no functional antheridium in this fungus and the ascogonium, therefore, does not receive a male nucleus from outside. It is, moreover, improbable that the second nucleus is derived from the adjoining stalk cell or from the trichogyne. The separating walls on both sides of the ascogonial cell are intact throughout, and consequently impassable to nuclei. Moreover, at the time when the ascogonial cell contains two nuclei, the trichogyne and the neighbouring stalk cell both possess their respective nuclei (Figs. 16, 17). There are, however, two other possible ways by which this binucleate condition may have arisen. Either the subterminal cell has possessed two nuclei from the time of the original segmentation of the archicarp, as is the case in *Thelebolus*, or the two nuclei arise by division of the pre-existing one. The former condition does not obtain here as the cells of the archicarp are strictly uninucleate when first formed. So the only possible mode of origin of the binucleate condition appears to be by division of the original nucleus, though this process has not actually been observed.

The next step in its development, after the appearance of the binucleate condition, is the further swelling of the archicarp. The resulting spherical body, now containing two nuclei, becomes covered with sterile hyphae (Figs. 18, 19), as already described, and forms the fruit body, whose diameter varies from  $10\mu$  to  $20\mu$  when fully matured.

*Ascus.* The most essential part of the perithecium is its spore-producing organ, the single ascus which at maturity completely fills it (Fig. 26). The development of ascogenous hyphae is entirely lacking in this fungus, and the whole process is simplified by the direct transformation of the ascogonial cell into the ascus, as in *Thelebolus*. The wall of the young ascus is thin, and is completely filled with protoplasm, in which are the two nuclei lying side by side. Subsequently they come close together, their clear vacuoles coalesce till both are within the same zone, and the two nuclei become completely fused together (Figs. 18–20) as in other ascomycetes. This fusion nucleus then increases in size (Fig. 21).



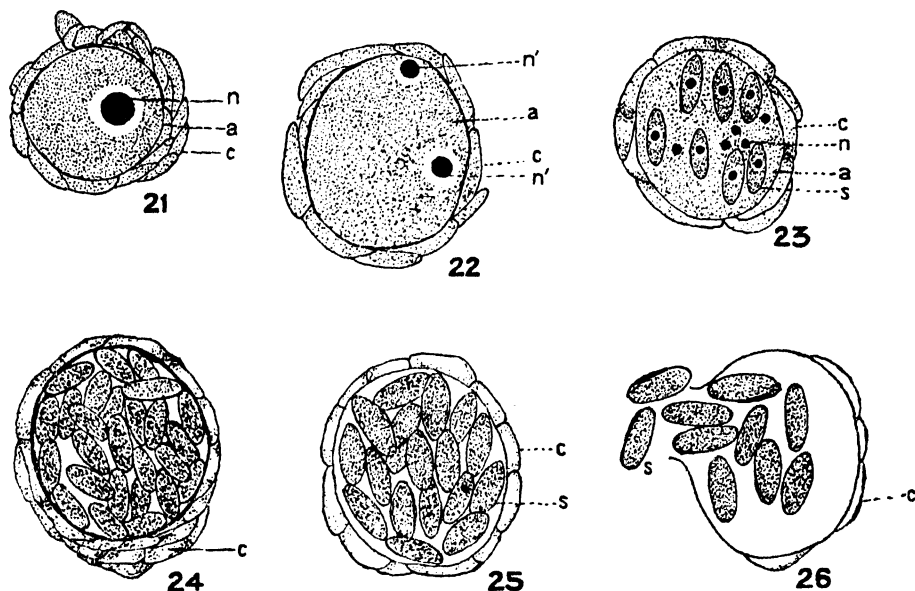
Almost at once the fusion nucleus undergoes division, and this is followed by three or four other divisions to form a number of daughter nuclei scattered throughout the ascus (Fig. 22). A portion of the cytoplasm collects around certain of the nuclei, and finally secretes a wall to form the young spore (Fig. 23). Detailed studies of different stages of division were not attempted owing to the small size of the nuclei. The mature asci contain a variable number of spores, which ranges from two to twenty-three. But, though it is a wide range, asci containing eight to twelve spores occur most commonly. The fact that asci can frequently be observed which contain fully formed spores, together with small immature ones and even free nuclei, indicates that the reduced number of spores is due to the failure of a varying proportion of the nuclei to take part in the formation of spores (Fig. 23). The mature asci are generally spherical in form with a thin wall (Fig. 24). The diameter of the fully matured ascus varies approximately from  $8\mu$  to  $19\mu$  in different individuals. In certain cases the spherical condition does not persist to the last, since they may become slightly oval.

The spores fill practically the whole cavity of the ascus. They are elliptical in form, with a very thin hyaline membrane, uniformly thickened throughout (Fig. 1). The membrane is smooth and homogeneous. The size of the spores varies considerably from  $5\mu$  to  $10\mu$  in length, and from  $3.5\mu$  to  $4.5\mu$  in width. The spores are uninucleate in the resting condition.

*Covering sheath.* Reference has already been made to the formation of a perithecial wall, but hitherto its detailed description has not been given. Stained preparations confirm the observations made on living material as to the origin of the hyphae from which it is formed. The first filament arises from the lowermost cell of the archicarp, coils round it, and becomes closely applied to the subterminal cell. This is followed by others which have a mixed origin, some from the same region from which the first one originates, and others from the vegetative mycelium from which the archicarp itself is formed. The hyphae are slender, composed of many cells, and occasionally branched. On originating from their respective parent hyphae, they follow the same course, from the bottom towards the top of the archicarp, which they encircle on all sides (Fig. 16), sometimes leaving out the tip of the trichogyne cell. Thus a covering sheath is formed around the young ascus, which is not at first compact in texture owing to the loose arrangement of the constituent hyphae (Fig. 19). Here and there these hyphae leave exposed small portions of the surface of the ascus, and in this sense the covering is at first incomplete.

In the young condition these covering filaments, except the first one, are not very closely applied to the young ascus. In a more advanced stage the ascus increases in size, and gradually presses its wall against the surrounding hyphae (Figs. 20-2).

At this stage the sheath may consist of two to three layers of hyphae, but the thickness is not uniform throughout (Fig. 21). In most of the cases examined, the number of hyphal layers may be reduced to one in the upper part of the perithecium, while the basal region of the same structure may



FIGS. 21-6. Fig. 21. The nucleus of the ascus enlarges preparatory to division, the covering hypha form an investment of one to three layers. Fig. 22. Ascus after first nuclear division. Fig. 23. Ascus with seven young spores and five superfluous nuclei. Fig. 24. Nearly mature perithecium, three-layered at the base, ascus wall distinguishable. Fig. 25. Mature perithecium. Fig. 26. Rupture of perithecium and exit of spores from ascus.

have two or three (Fig. 24). As the ascus enlarges it exerts pressure on the covering sheath from within, and as a consequence the constituent filaments become closely compacted.

In very old perithecia, the sheath is extremely modified. The hyphal cells lose their contents and the walls get much thicker and faintly yellowish in colour. Ultimately the inner layers disappear as the perithecium ripens and only the single outer layer remains. It surrounds the ascus wall as a dead tissue of dry cells with thick walls devoid of contents (Fig. 25). During the whole course of its development the outer surface of the sheath does not possess any appendages. It is always smooth except in certain cases where the free apices of some hyphae may be found projecting in different directions. The reduction in number of hyphal layers is doubtless due to degeneration of some of the cells, but the sheath does not show any differentiation in structure, as for example in *Eurotium*, where two distinct regions are visible, the inner nutritive and the outer protective.

The spores when young are embedded in the remaining cytoplasm of the ascus which acts as a cement to bind all of them together. But when

they are fully matured this surrounding cytoplasm is gradually diminished and the spores form a group practically filling the whole cavity of the ascus (Figs. 24, 25). At the time of their liberation the wall of the perithecium breaks down either completely, or partially, towards its upper region. The wall of the ascus, which is now exposed, opens by a split through which the spores come out (Fig. 26). There is no definite manner in which this splitting may occur, nor is there any particular spot for it, except that it generally takes place towards the upper part of the perithecium. The perithecia when put in water swell, but the spores are not discharged by explosion as in so many other ascomycetes. The spores were in some cases found to germinate when they were still inside the perithecium.

#### DISCUSSION.

The main interest of this fungus lies in the relative simplicity of its reproductive structures. From a simple spiral archicarp there is formed without fertilization a single ascus which becomes invested with sterile hyphae to form a small completely closed perithecium of the type familiar in the lower members of the Plectascineae.

The archicarp, which is at first a uninucleate branch, develops into a spiral coil or scolecite of a type found in many members of the lower families of both the Plectascineae and Pyrenomycetinae. Such structures have been described in *Penicillium* (3), *Aspergillus* (4, 9), *Chaetomium* (7), *Sordaria* (5), and others.

It is, when mature, differentiated into three regions, a multicellular stalk, a unicellular ascogonium, and a trichogyne. It very closely resembles the archicarps of *Aspergillus herbariorum* (4) and *A. repens* (9). But whereas in both these forms all the segments of the archicarp are multinucleate, in the present form these cells contain a single nucleus. In this respect it more closely resembles the archicarp of some of the simplest forms of the Sphaeriales, for example, *Chaetomium globosum*.

In *A. herbariorum* (4) and in *Penicillium vermiculatum* (3) an antheridium fuses with a cell of the archicarp, although in neither form has an actual transference of male nuclei been observed. In the present case a branch arises, which in its behaviour and general relations to the archicarp might well be regarded as an antheridium. It never functions as such, and eventually takes part in the formation of the wall of the perithecium. At a later stage, however, the ascogonium is binucleate and the mode of origin of this condition has been carefully considered. The possibility of its being a male nucleus from the antheridium may be at once excluded.

Migration of a nucleus from one of the neighbouring cells of the stalk or from the trichogyne is a possibility which observation has failed to confirm. The separating walls are in every case intact, and whereas the adjacent stalk cell invariably contains a nucleus, the nucleus of the trichogyne

can be seen to degenerate at a later stage. The only remaining possibility is that the binucleate condition is the result of a division of the original nucleus of the ascogonium, though this has not actually been observed.

At this stage the archicarp consists of a row of cells, all of which are uninucleate except the subterminal cell which is binucleate. This is strongly reminiscent of the condition described by Ramlow in *Thelebolus stercoreus* (11). But here this condition is brought about by the subdivision of a multinucleate hypha and the young ascus is thus binucleate from the first.

The young ascogonium contains, therefore, two nuclei, both of which must be regarded as the product of the division of the pre-existing nucleus and therefore presumably both of the same kind. Since these nuclei are destined to fuse together in the young ascus, we are dealing with a reduced form of sexuality, or homiogamy resembling that of *Humaria granulata* (2) and *Lachnea stercorea* (1).

In the majority of the lower ascomycetes, where an ascogonium exists, it eventually undergoes division into segments from which the ascogenous hyphae arise. But in the present form the ascogonium behaves like that of *Thelebolus* (11) in the *Ascobolaceae*, and develops directly into an ascus. Ascogenous hyphae are consequently entirely lacking, a character which may be regarded as indicating the survival of a primitive condition, but may equally be the result of regressive simplification.

Fusion of nuclei which here, as in all ascomycetes, takes place in the ascus and precedes the divisions which result in the formation of the spores is thus the only nuclear fusion in the life-history. In this respect it resembles *Thelebolus*. It is to be regretted that the small size of the nuclei in this form renders them unsuitable for cytological observation. The normal three successive nuclear divisions are here followed by a fourth and a fifth, resulting in an ascus with thirty-two nuclei.

*Systematic position.* The general structure of the fruit body clearly indicates the affinity of this species to the lower families of the Ascomycetes. The closed perithecium is essentially of the type found in the *Perisporiales* and in the *Aspergillaceae*. Although certain resemblances to *Thelebolus* have been pointed out, notably the development of the single ascus from a binucleate ascogonium, and the multispored ascus, these resemblances are not necessarily significant. It is, in fact, uncertain, in the absence of detailed information of the development of other species of *Thelebolus* and *Rhyarobius*, whether the fertile filament of *Thelebolus stercoreus* is indeed an archicarp. Moreover, the structure of the mature ascus in *Thelebolus* and the complex character of its enveloping sheath in no way suggest a relation to the species under consideration.

There are, on the other hand, only two respects in which the development of the perithecium in this fungus differs from that of *Aspergillus*, viz.

the uninucleate ascogonium and the absence of ascogenous hyphae. These differences together with the minute size of its perithecium appear to be of sufficient importance to warrant its separation from that genus, but it may possibly be accepted as the end member of a reduction series in the Aspergillaceae, occupying in that family a position analogous to that of *Sphaerotheca* in the Erysiphaceae. It may be recalled that some species of *Eurotium* have also been described which lack conidia.

*Microeurotium*, nov. gen.

Mycelium bene evolutum, septatum. Cleistothecia minuta. Peridium tenue, laeve, hyalinum. Asci solitarii. Sporidia 2 vel  $\infty$ . Conidia non vidi.

*Microeurotium albidum*, nov. sp.

*Mycelio* albo vel pallide flavescente; *cleistotheciis* subflavis vel hyalinis, pellucidis, sphericis, 10–20  $\mu$  diam.; *ascis* tenuibus, irregulariter dehiscentibus, 2–23 sporis; sporidiis ellipticis, laevibus, hyalinis, 5–10  $\mu$  long., 3.5  $\mu$ –4.5  $\mu$  lat. (magna pro parte 8  $\mu$  long., 4  $\mu$  lat.).

*Hab.* in stercore (?).

#### SUMMARY.

1. The spores on germination produce a septate mycelium. The segments are at first multinucleate but by later subdivision form uninucleate cells in the older hyphae.

2. A spiral archicarp arises as a lateral branch from a vegetative hypha. At first uninucleate it is later divided into a two- to three-celled stalk, an ascogonium, and a trichogyne.

At this stage, all the cells are uninucleate; but later, the ascogonium contains two nuclei which probably are formed by division of the pre-existing one.

3. There is no functional antheridium present, but a sterile branch arises from the basal cell of the stalk, which coils round the ascogonium cell and, with other later formed hyphae, gives origin to the wall of the perithecium.

4. The trichogyne degenerates and the subterminal cell enlarges to form a spherical ascus. The two nuclei in the young ascus fuse together. The definitive nucleus by repeated division forms sixteen to thirty-two nuclei, only a proportion of which develop into spores; the number of spores varies from two to twenty-three in different asci. The spores are uninucleate.

5. The covering filaments enclose the ascus and form the wall of the perithecium. The wall is at first loose but ultimately becomes compact and closely applied to the ascus wall. It is at first one to three layers thick, but in the mature perithecium is generally reduced to one.

6. In the fully matured perithecium, the wall breaks down and the spores are liberated by the rupture of the ascus wall which may take place in any part.

7. The fungus is referred to the Aspergillaceae and is held to form the type of a new genus related to *Aspergillus*.

In conclusion I desire to express my thanks to Mr. J. R. Ramsbottom who kindly drew up the Latin diagnosis, and to Assistant-Professor R. J. Tabor who handed over to me his original cultures and helped and advised me during the progress of the work.

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## NOTE.

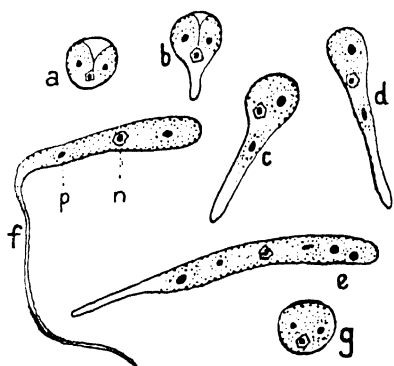
**NOTE ON THE GERMINATION OF THE ZYGOTE OF *ULOTHRIX RORIDA* THURET.**—In a previous paper<sup>1</sup> an account was given of the life-history of *U. rorida* Thuret in which it was shown that gametes are produced only in the spring and fuse in pairs to form rounded zygotes (*a*). Following gamete formation the growth of the alga ceases and is renewed in the autumn.

An attempt has been made to follow the development of the zygote during this period. Material producing gametes was brought into the laboratory in the morning and by the next day numerous zygotes were found forming bright green patches on coverslips placed at the bottom of the dish. After two days the coverslips with their zygotes were removed and placed in washing tubes closed at both ends with bolting silk. The tubes were left in a natural stream and at intervals of a week the coverslips were removed and the germinating zygotes fixed and stained.

Within two days most of the zygotes had put out a small protuberance (*b*) which grew gradually in length at the expense of the rounded part of the zygote, until after about a week the zygotes had enlarged and taken the form of elongated structures (*d*), more or less uniformly green, and containing two pyrenoids and one nucleus. By the end of a fortnight these elongated bodies had developed long, fine, colourless attachment organs (*e*) and in many cases pyrenoids had fragmented. After one month the attachment organs had elongated further (*f*), but otherwise there was little change.

Mortality was high among the zygotes and it was not possible to follow their development after six weeks from germination. Up to this time the bodies were without any thickened membrane and the nucleus was undivided. On each coverslip were to be found some zygotes (*g*) which had not germinated but were still healthy and undivided.

Dodel<sup>2</sup> describes somewhat similar early stages in the germination of the zygote of *U. zonata* and states that after a resting period of several months the zygote resumes growth and finally divides into zoospores. Gross<sup>3</sup> describes



*U. rorida*. Stages in the germination of the zygote. (*a*) 2 days, (*b*) 5 days, (*c*) 8 days, (*d*) 10 days, (*e*) 15 days, (*f*) 1 month, (*g*) ungerminated. (*p*) pyrenoid, (*n*) nucleus.  $\times 500$ .

<sup>1</sup> Lind, E. M., A Contribution to the Life-history and Cytology of Two Species of *Ulothrix*. Ann. Bot., xlv. 1932.

<sup>2</sup> Dodel, A., Jahrbuch Wiss. Bot., x. 1876.

<sup>3</sup> Gross, I., Entwicklungsgeschichte, Phasenwechsel und Sexualität von *Ulothrix*. Archiv f. Protistenk. Bd. 72, Heft 2.



reduction division of the diploid zygote nucleus very shortly after the fusion of the gamete nuclei. Klebs<sup>1</sup> found germination to occur in sugar solution after one month ; and the zygote, still rounded, developed into two or four spores, each of which gave a sporeling. He did not observe an attachment organ.

Although the final fate of the zygote of *U. vorida* after the resting period has not been ascertained, it seemed worth while to record these observations on the early stages of germination.

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<sup>1</sup> Klebs, Bedingungen der Fortpflanzen bei einigen Algen und Pilzen. Jena. 1896.





I. A. R. I. 75.

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